Data Evaluation Report on a Pilot Study of Larval *R. clamitans* Response to Atrazine Exposure in Terms of Metamorphosis, Gonadal and Laryngeal Morphology and Selected Hormonal and Enzymatic Activities.
EPA MRID Number 458677-03

Data Requirement::

EPA DP Barcode D288775

EPA MRID 458677-03
EPA Guideline 70-1(Special Study)

Test material: **Purity:** 97.1%
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,
U. S. Environmental Protection Agency

Secondary Reviewer(s): Joseph E. Tietge, M.S., Research Aquatic Biologist **Date:**
Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory (Duluth),
U. S. Environmental Protection Agency

Stephanie Irene, Ph.D., Senior Advisor **Date**:
Environmental Fate and Effects Division, ERB 3, U. S. Environmental Protection Agency

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: 05/31/2003

CITATION: Hecker, M., K. K. Coady, D. L. Villeneuve, M. B. Murphy, P. D. Jones and J. P. Giesy. 2003. A Pilot Study of Response of Larval *Rana clamitans* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology and Selected Hormones and Enzyme Activities. Aquatic Toxicology Laboratory, Michigan State University, National Food Safety and Toxicology Center, E. Lansing, MI. Sponsor: Syngenta Crop Protection, Inc., Laboratory Study ID ECORISK Number MSU-03.

EXECUTIVE SUMMARY:

Green frog (*Rana clamitans*) tadpoles reared from field-collected eggs were exposed for 273 days, beginning 5-days post-hatch, to two concentrations (10 and 25 µg/L) of atrazine. Positive controls, dihydroxytestosterone and 17-β estradiol (0.1 µg/mL in 0.005% ethanol), a negative control (water) and a solvent control (0.005% ethanol) were also run. Replicates (9) consisted of 30 free-swimming tadpoles each. Initially,(exposure days 0 - 67) animals were maintained under static renewal conditions in 4 L of test solution; 50% tank changes were conducted every 72 hours. From Days 68 to Day 273, tadpoles were maintained in tanks containing 16L of test solution under static renewal. After 273 days, exposures were terminated and tadpoles were maintained in continuous flow-through 10-L glass tanks housed in large acrylic tanks containing 80 L of continuously renewing freshwater. At metamorphosis (fore-limb emergence), tadpoles were either housed individually or in small groups in 10-L glass tanks containing approximately 500 mL of freshwater. Over the study period, mortality across all treatment groups averaged 76.5% and was attributed to poor water quality and overcrowding during the 273- day static-renewal phase of the study. While mean-measured concentrations of atrazine were relatively consistent with nominal values, measurements were made on freshly prepared stock solutions; hence it is unclear what atrazine concentrations were present in aged exposure solutions. Additionally, measurable levels of atrazine were detected in the negative controls. Although the concentrations of positive control hormones were not measured, the positive controls using dihyrdotestosterone and 17-β estradiol suggested that green frogs only reacted to androgenic chemicals resulting in predominately (97.6%) male frogs, while the frogs were not affected by estradiol. It is uncertain whether this means that green frogs are unresponsive to estrogenic chemicals, or whether there was sufficient estradiol in solution to elicit an effect. While no intersex (testicular and ovarian tissue in the same animal) was observed in any of the treatment groups, this observation was based on gross morphology, and apparently there were difficulties in discerning the presence of gonads using this process. While time to and age at metamorphosis and the size of metamorphs were reduced in frogs treated with 10 µg/L atrazine, there was no difference in these same parameters for frogs treated with 25 µg/L atrazine relative to negative controls. Although there were no dose dependent effects in green frogs related to atrazine treatment, only two concentrations were monitored. Additionally, because only a limited number of frogs survived to complete metamorphosis, the conclusions regarding sex ratio data are questionable.

No analysis of gonad histology is provided and no measurements were made of aromatase levels. Contrary to the GLP statement, this study represents an interim report and not a final report. .

The high mortality indicative of poor water quality and overcrowding and the lack of response to the positive estradiol control made it difficult for the study authors to test the hypothesis that atrazine exposure was associated with developmental effects in amphibians. The study did provide the authors with a better appreciation for the conditions under which green frogs should be housed, and it suggests that the green frog may not be adequate for examining the effects of atrazine on amphibian development..

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 Stanadard 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 Inspections were conducted.
- Final Report was written
- Raw data, documentation, records, protocols, and final report was archieved.

A. MATERIALS:

1. Test Material Atrazine

Description: Not reported

Lot No./Batch No. : Not reported

Purity: 97.1%
Stability of compound under test conditions: Not reported

Storage conditions of test chemicals: Not reported

2. Test organism:

Species: Green frog (*Rana clamitans*)

Age at test initiation: Larvae (Gosner Stage 25; approximately 5 days post-hatch
Weight at study initiation: (mean and range)
Length at study initiation: (mean and range)

Source: Eggs field-collected as a single mass of fertilized eggs from Giesy pond in Williamston, MI (7/10/2001)

B. STUDY DESIGN:

Objective: 1) To develop and validate methods of husbandry and exposure for conducting laboratory studies with *R. clamitans*.
2) To determine the response of larval *R. clamitans* to atrazine by assessing metamorphosis and reproduction indices when animals are exposed during larval development. Indices to be evaluated include:

- % initiating metamorphosis
- % completing metamorphosis
- time to metamorphosis
- fresh post-mortem body weight and snout-vent length
- incidence of gross gonadal abnormalities
- histology of the gonads.

1. Experimental Conditions

a) Range-finding Study: Current study represents a pilot study

b. Definitive Study

Table 1 . Experimental Parameters

Parameter	Details
Acclimation: period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 8 days transitioned from pond to laboratory water over unspecified time period not reported
Duration of the test	506-day study of which 273 days exposed to test solutions
Test condition static/flow- through Type of dilution systemfor flow-through method. Renewal rate for static renewal	 static renewal NA 50% test solution change every 72 hours
Aeration, if any	NA

Material: (glass/stainless steel) Size: Fill volume:	glass 10 L 4 L After 67 days of exposure, tadpoles transferred from 10L tanks to tanks containing 16 L of test solution. After 273 days, tadpoles transferred to continuous flow-through system of clean freshwat through a 10-L glass tank housed in large acrylic tanks containing 80 L of continuously renewing freshwater; once animals initiated metamorphosis (fore-limb emergence), removed from flow-throug system and housed as individuals or small groups in 10-L glass tanks containing approximately 500 ml of freshwater
Source of dilution water Quality:	Treated well water (MSU-University Research Containment Facility
Hardness pH Dissolved oxygen Total organic carbon Particulate matter Ammonia Nitrite Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	426 mg/L as $CaCO_3$ (static); 7.87 (static); 8.0 mg/L (static); 6.1 mg/L (flow-through) 0.04 mg/L (static); 0.02 mg/L (flow-through) (see reviewer's comments) 0.22 mg/L (static); 0.02 mg/L (flow-through) 21.3°C (static); 24.8°C (flow-through) NA

Parameter	Details
Number of replicates/groups: negative control: water solvent control: 0.005% ethanol treated ones: atrazine at 10 and 25 µg/L positive controls: dihydroxytestosterone and estradiol	 9 9 9 + 9 9 + 9
Number of organisms per replicate /groups: control: solvent control: treated ones:	 (30 tadpoles /rep) x 9 reps = 270 tadpoles 30 tadpoles /rep) x 9 reps = 270 tadpoles 30 tadpoles /rep) x 9 reps = 270 tadpoles
Biomass loading rate	30 tadpoles/4 L → 30 tadpoles/10 L
Test concentrations: nominal: measured:	 10 and 25 µg/L 13.8 and 28.1 µg a.i./L
Solvent (type, percentage, if used)	freshwater for atrazine; 0.005% ethanol for positive hormone controls
Lighting	not reported
Feeding	Appendix reports that frog brittle was analyzed yielding inconclusive results. Feeding regime is not reported
Recovery of chemical Level of Quantitation Level of Detection	ELISA (Envirogard Triazine®; Strategic Diagnostics Newark, DE)/Beacon Analytical triazine plate (Beacon Analytical Systems, Portland, ME) LOD 0.025 µg/L (Envirogard); 0.05 µg/L (Beacon)
Positive control {if used, indicate the chemical and concentrations}	dihydroxytestosterone 0.1 µg/L 17-β estradiol 0.1 µg/L both hormones in 0.005% ethanol
Other parameters, if any	NA

2. Observations:

Table 2: Observations

Criteria	Details
Parameters measured including the sublethal effects/toxicity symptoms	mortality; time to metamorphosis, number completing metamorphosis, age (days) at metamorphosis, length, weight, gonadal abnormalities, sex
Observation intervals	daily
Were raw data included?	
Other observations, if any	

Animals not reaching metamorphosis by 506 days were sacrificed.
All frogs completing metamorphosis were analyzed for gross morphology and histology of the gonads (no mention of kidneys).

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.]

Exposures were initiated at 5 days post-hatch when tadpoles were free swimming and external gills were resorbed (Gosner stage 25). The feeding regime for the tanks was not discussed

Atrazine levels [of stock solutions] were measured following static renewal and therefore represented fresh as opposed to aged exposure solution values. Measurements were made using two different ELISA kits and yielded roughly similar exposure estimates (Table 3) over the course of the study. In general, mean-measured concentrations ranged from 112% to 159% of nominal. Atrazine was detected in the controls at concentrations that were within the LOQ (0.025) for the assay. Because data was not reported for the solvent control or either of the positive controls, it is not clear whether the contamination was limited to negative controls or across all treatments. Triazine ELISA kits did not arrive within the first 60 days of exposure, and it is unclear whether Syngenta was verifying exposure at this time or whether the study was based strictly on nominal concentrations during the first 60 days. Dead tadpoles were partially degraded, partially eaten and/or covered in fungus when discovered dead and therefore many of them could not be salvaged for later analysis. These results suggest poor tank conditions for supporting such rapid deterioration of the tadpoles.

No measurements were recorded for dihydroxytestosterone or estradiol in the positive controls.

Table 3. Nominal versus mean-measured atrazine concentrations.

Treatment	Atrazine (nominal) µg/L	Syngenta mean-measured µg/L	MSU mean-measured µg/L
Control	0	0.14 (0.07 - 0.23)	0.10 (0.06 - 0.17)
10 µg/L	10	15.91 (12.03 - 19.90)	11.76 (10.21 - 13.65)
25 µg/L	25	27.95 (24.92 - 31.24)	28.23 (25.14 _ 31.60)

Across all treatment groups, mortality averaged 76.5% (**Table 4**). Mortality was reported to be greatest during the first month of the exposure period and decreased as tadpoles grew older. According to the report, "mass mortality events occurred early in the study and usually occurred within a time span of 24 hours." Mortality rates declined after 273 days when tadpoles were transferred out of static renewal into flow-through water system. Although there was no significant difference in mortality between atrazine-treated and negative controls, there was a difference between the positive control treatments and the ethanol solvent control; the dihydroxytestosterone group had significantly fewer deaths. High mortality rates were potentially attributed to high ammonia levels in the static renewal systems.

By exposure Day 58, ammonia (NH_3) concentrations were between 0.8 - 0.9 mg/L and nitrite (NO_2) concentrations were as high as 3.0 mg/L

Because of the loss of so many animals, hormone concentrations were not analyzed as an endpoint in this study.

Table 4 . Average percent mortality for each treatment group over 506 day study period.

Treatment	Average % Mortality
Untreated Controls	79.2
Ethanol Control	74.8
Dihydroxytestosterone	62.7
17-β estradiol	85.7
10 µg/L	73.1
20 µg/L	83.3

The first initiation of metamorphosis was observed on exposure day 99 and the first completion of metamorphosis was observed on day 112. As of day 143, 10 tadpoles had completed metamorphosis. Between

day 143 (December 7) and 285 (April 28), no tadpoles had initiated metamorphosis. Age at initiation and completion of metamorphosis was significantly different among the atrazine-treated groups and the untreated controls; frogs treated with 10 µg/L atrazine initiated and completed metamorphosis at a significantly older age compared to both untreated control frogs and frogs exposed to 25 µg/L **(Table 5**). Frogs in the estradiol treatment initiated metamorphosis at a significantly younger age as compared with both the ethanol control and frogs exposed to DHT.

Frogs treated with 10 µg/L atrazine were significantly shorter (SVL) than frogs in the 25 µg/L atrazine exposure group; however, there were no significant differences in weight between any of the treatment groups at metamorphic completion (**Table 6**).

The incidence of gross gonadal deformities ranged from 0 to 5.9% across all treatments with size incongruity between gonad pairs as the most commonly observed anomally. No intersex gonads (testicular and ovarian tissue within the same individual) were observed during gross inspections. In two frogs, gonad or gonad pairs could not be located in both the estradiol and DHT treatments.

Sex ratios in the atrazine and untreated controls did not differ significantly from the expected 50:50 male:female ratio **(Table 7**). While estradiol and ethanol control sex ratios did not differ from a ratio of 50:50, the DHT treated animals were 97.7% male.

Table 5. Number of green frogs surviving to and completing metamorphosis.

Treatment	Initial N	# Frogs Initiating Metamorphosis	# Frogs Completing Metamorphosis
Untreated Controls	285	58	44
Ethanol Controls	280	69	47
Dihydroxytestosterone	291	104	75
17-β estradiol	282	40	33
10 µg atrazine/L	292	77	64
25 µg atrazine/L	292	48	37

Table 6. Mean ages (days), lengths (cm), and weights (g) at metamorphosis for *R. clamitans*.

Treatment	Mean age at initiation	Mean age at completion	Mean Weight (g)	Mean Length (cm)
Untreated Controls	328.14	336.75	1.76	2.52
Ethanol Controls	349.99	359.68	1.56	2.47
Dihydroxytestosterone	350.48	368.53	1.50	2.42
17-β estradiol	329.73	342.15	1.64	2.57
10 µg atrazine/L	361.81	376.70	1.48	2.39
25 µg atrazine/L	335.27	342.14	1.64	2.54

Table 7. Percent male and female green frogs in each treatment.

Treatment	% Males	% Females
Untreated Controls	43.1	56.9
Ethanol Controls	47.4	50.9
Dihydroxytestosterone	97.7	2.3
17-β estradiol	36.8	63.2
10 µg atrazine/L	40.3	59.7
25 µg atrazine/L	40.9	59.1

C. REPORTED STATISTICS: Kolmogrov-Smirnov's One Sample test with Lillifor's transformation was used to assess whether or not the data sets were normally distributed. When normally distributed, ANOVA followed by Fisher's LSD was used to detect significant differences between treatment groups. For non-normally distributed data, non-parametric Kruskal-Wallis test/Mann-Whitney U Test was used. The Chi-square test was used to detect differences in expected sex ratios and Pearson's Chi-square was used to test for differences in the incidences of gross gonadal abnormalities.

D. VERIFICATION OF STATISTICAL RESULTS: Statistical analyses run using SAS® (Statistical Analysis System, Release 8.01, Cary, North Carolina); see attached output.

E. STUDY DEFICIENCIES: The feeding regime was not reported; however, the animals were apparently fed frog brittle. The appendix reports that a previous analysis of the food was "inconclusive". It is unclear what "inconclusive" refers to; however, an analysis of the food supply was apparently not run

Atrazine was detected in the negative control.

Water quality during the static renewal phase of the study was poor.

F. REVIEWER'S COMMENTS:

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

A major problem in this study is the low survival rate which ranged from 37-14%. Although the report correctly notes that the rate of mortality decreased after the first 30 days, it was still substantial. For example, control mortality (as estimated from Figure 1) at 30 days was about 80 individuals. Mortality in the controls for the remainder of the test was about 120 individuals. This high mortality rate indicates severely inadequate methods and suggests that the study may not be useful..

The high mortality rates across all treatments coupled with data showing high ammonia/nitrite levels suggest that this study was probably compromised by poor water quality caused by overcrowding in a static renewal system. Because only 50% of the water was changed every 72 hours for the first 67 days of exposure, there is a high potential for waste products to accumulate. . The authors acknowledged that high mortality was probably caused by tadpole overcrowding in static tanks and that poor water quality (high ammonia and nitrite) may have contributed to mortality. The authors also acknowledged that these factors may have delayed growth and development of tadpoles because increased rates of development coincided with a shift from static to flow-through exposure systems. Tadpoles that underwent metamorphosis early tended to come from tanks that had experienced high mortality rates during the first month of exposure and were therefore subject to less crowded conditions. Although the authors stated that the differences in time to complete metamorphosis between treatment groups appeared to be a result of tank effects on relative growth rates rather than atrazine treatment; it may be more precise to conclude that tank effects obscured the study's ability to detect treatment effects. Given the confounding tank effects, it isn't possible for the authors to conclude that exposure to 10

and 25 µg/L atrazine does not consistently affect age, length, or weight of *R. clamitans* at metamorphic completion.

Because only about 24% of the tested organisms completed metamorphosis, and all of the analyses were conducted on juvenile organisms, the sampling strategy may have been biased and did not represent the population in the test.

While dihydroxytestosterone-treated frogs were identified as predominately (97.7%) male, the estradiol-treated frog sex ratio did not differ significantly from 50%. It is unclear whether the estradiol treatments, as a positive control, should have skewed sex ratios in favor of females; however, it is clear that the "treatment" did not impact sex ratios. Because hormone levels in the positive control were not measured, it is uncertain whether the lack of responsiveness is due to insufficient stimulus, poor water quality issues, or insensitivity of green frogs to estradiol treatments (i.e., green frogs represent a poor species for testing estrogenic responses). The authors stated that green frogs are not feminized when exposed to exogenous estradiol, but rather they are masculinized when exposed to exogenous androgens (*e.g.* DHT) and cite Foote and Witschi 1939. The fact that estradiol did not affect gonadal differentiation is inconsistent with previous studies, and it is not known if the frogs in this study were exposed to an efficacious dose of the hormone. .. In another study conducted by the same laboratory, estradiol concentrations in a static renewal system were less than 10% of the nominal target concentration. As a consequence, they did not observe the expected feminizing effects on *X. laevis*. This study with green frogs did not analyze estradiol concentrations, but they were certainly substantially below the target concentration given the static-renewal exposure regimen used.

According to Hayes (1998), estradiol treatment of *R. clamitans* did not affect sex ratio or produce mixed results (no effect on sex ratio to effects favoring either males or females); treatment of Ranids with testosterone produced primarily males.

Green frogs are a less studied experimental model than *X. laevis*. In *X. laevis*, the period of sensitivity toward femininization is during early prometamorphosis. This study was conducted in a manner that included the presumptive sensitive period of this species (*i.e.*, prometamorphosis).

Apparently there was some difficulty in identifying the presence of gonads in some of the animals, suggesting that the accuracy in detecting gonadal anomalies based on visual examinations (gross morphology) was somewhat limited.

The overall hypothesis tested was that waterborne concentrations of atrazine would not have an adverse effect on the gonads of the green frog (*Rana clamitans*) when exposed during the critical phases of development.

Based on an analysis of the raw atrazine measured concentration data (see attached SAS© [Statistical Analysis System, Release 8.01, Cary, North Carolina] and although only a limited number of analyses are reported on tank solutions, the tank atrazine concentrations ranged from 116 to 347% of mean-measured concentrations in stock solutions. On average, mean-measured concentrations (stock and tank solutions combined) contained 0.10 ± 0.016 µg/L, 11.76 ± 4.87 µg/L and 28.23 ± 8.47 µg/L in 0, 10 and 25 µg/L nominal exposure groups. Based on analyses conducted by Syngenta, exposure solutions averaged 0.14 ± 0.20 µg/L, 15.9 ± 6.7 µg/L, and 27.9 ± 8.88 µg/L. Although both sets of analyses tended to agree with one another, theyindicated that atrazine was present in the dilution water control and at levels that other studies have shown to result in gonadal

developmental effects (Hayes *et al.* 2002*a* and 2002*b*).

Previous studies conducted by Hayes *et al.* 2002*a*,b showed effects as low as 0.1 µg/L; however, this study only used 10 and 25µg/L exposure levels. Also, Hayes' studies suggest that the incidence of gonadal effects was higher at lower doses. The effect on delayed time to metamorphosis and smaller size of metamorphs treated with 10 µg/L relative to both controls and animals treated with 25 µg/L may be reflective of a similar pattern.

G. CONCLUSIONS: Green frog (*Rana clamitans*) tadpoles reared from field-collected eggs were exposed for 273 days, beginning 5-days post-hatch, to two concentrations (10 and 25 µg/L) of atrazine. Positive controls, dihydroxytestosterone and 17-β estradiol (0.1 µg/mL in 0.005% ethanol), a negative control (water) and a solvent control (0.005% ethanol) were also run. Replicates (9) consisted of 30 free-swimming tadpoles each. Initially (exposure days 0 - 67) animals were maintained under static renewal conditions in 4 L of test solution; 50% tank changes were conducted every 72 hours. From Day 68 to Day 273, tadpoles were maintained in tanks containing 16L of test solution under static renewal. After 273 days exposures were terminated and tadpoles were maintained in a continuous flow-through 10-L glass tanks housed in large acrylic tanks containing 80 L of continuously renewing freshwater. At metamorphosis (fore-limb emergence) tadpoles were either housed individually or in small groups in 10-L glass tanks containing approximately 500 mL of freshwater.

Over the study period, mortality across all treatment groups averaged 76.5% and was attributed to poor water quality and overcrowding during the 273 day static-renewal phase of the study. While mean-measured concentrations of atrazine were relatively consistent with nominal values, measurements were made on freshly prepared stock solutions; hence it is unclear what atrazine concentrations were present in aged exposure solutions. Additionally, measurable levels of atrazine were detected in the negative controls. Although the concentrations of positive control hormones were not measured, the positive controls using dihydrotestosterone and 17-β estradiol suggested that green frogs only reacted to androgenic chemicals resulting in predominately (97.6%) male frogs, while the frogs were not affected by estradiol. It is uncertain whether this means that green frogs are unresponsive to estrogenic chemicals or whether there was sufficient estradiol in solution to elicit an effect. While no intersex (testicular and ovarian tissue in the same animal) was observed in any of the treatment groups, this observation was based on gross morphology and there were apparently difficulties in discerning the presence of gonads at all using this process. While time to and age at metamorphosis and the size of metamorphs were reduced in frogs treated with 10 µg/L atrazine, there was no difference in these same parameter for frogs treated with 25 µg/L atrazine relative to negative controls. Although there were no dose-dependent effects in green frogs related to atrazine treatment, only two concentrations were monitored.

Contrary to the GLP statement, this study represents an interim report and not a final report.

The high mortality indicative of poor water quality and overcrowding and the lack of response to the positive estradiol control made it difficult for the study authors to test the hypothesis that atrazine exposure was associated with developmental effects in amphibians. The study did provide the authors with a better appreciation for the conditions under which green frogs should be housed, and it suggests that the green frog may not be adequate for examining the effects of atrazine on amphibian development. The high mortality indicative of poor water quality and overcrowding and the lack of response to the positive estradiol controlmake it difficult to believe that this study was a sensitive indicator of the potential effects of atrazine on green frogs.

H. REFERENCES:

Foote, C. L. and E. Witschi 1939. Effect of sex hormones on the gonads of frog larvae (*Rana clamitans*): sex inversion in females; stability in males. The Anatomical Record 75(1): 75 - 83.

Hayes, T. B. 1998. Sex determination and primary sex differentiation in amphibians: genetic and developmental mechanisms. Journal of Experimental Zoology 281: 373 - 399.

Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. S. Stuart, and A. Vonk. 2002a. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences of the United States of America 99(8): 5476 - 5480.

Hayes, T. B., K. Haston, M. Tsui, A. Hoang, C. Haeffele, and A. Vonk. 2002b. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environmental Health Perspectives.

AVERAGE MEAN MEASURED CONCENTRATION ACROSS TREATMENTS 1265

Obs	CONC	_TYPE_	_FREQ_	MEAN	STD	CV
1	0	0	30	0.1016	0.16001	157.557
2	10	0	28	11.7594	4.87077	41.420
3	25	0	27	28.2307	8.47165	30.009

PERCENT (PERC) OF ATRAZINE IN TANK RELATIVE TO STOCK SOLUTIONS 1266

Obs	CONC	_TYPE_	_FREQ_	STOCK	TANK	PERC
1	0	0	2	0.0872	0.3029	347.488
2	10	0	2	11.2688	18.1366	160.945
3	25	0	3	27.7255	32.2723	116.399

AVERAGE SYNGENTA MEAN MEASURED CONCENTRATIONS OF ATRAZINE 1267

Obs	CONC	_TYPE_	_FREQ_	MEAN2	STD	CV
1	0	0	18	0.1418	0.19528	137.680
2	10	0	10	15.9100	6.68937	42.045
3	25	0	15	27.9091	8.85104	31.714

COMPARISON OF MSU VERSUS SYNGENTA-MEASURED ATRAZINE CONCENTRATIONS AND PERCENTAGE RELATIVE 1268

Obs	CONC	MEAN	MEAN2	PERC
1	0	0.1016	0.1418	71.604
2	10	11.7594	15.9100	73.912
3	25	28.2307	27.9091	101.152

AVARAGE PERCENTAGE OF MALES AND FEMALES ACROSS ALL TREATMENTS (ACTUAL TREATMENTS NOT LISTED 1269

Obs	_TYPE_	_FREQ_	MALES	FEMALES	STD_M	STD_F
1	0	54	0.50648	0.49121	0.31086	0.30996

AVERAGE PERCENTAGE OF MALES BY TREATMENT GROUP 1052

Obs	GROUP	_TYPE_	_FREQ_	MEAN	STD	CV
1	10	0	9	38.9889	14.1583	36.3138
2	25	0	9	36.9388	20.4962	55.4870
3	CONTR	0	9	44.7173	32.7762	73.2965
4	DHT	0	9	98.2194	3.5370	3.6011
5	E2	0	9	35.6803	25.4366	71.2905
6	ETOH	0	9	41.2676	24.8757	60.2791

AVERAGE PERCENTAGE OF FEMALES BY TREATMENT GROUP 1053

Obs	GROUP	_TYPE_	_FREQ_	MEAN	STD	CV
1	10	0	9	61.0111	14.1583	23.206
2	25	0	9	63.0612	20.4962	32.502
3	CONTR	0	9	55.2827	32.7762	59.288
4	DHT	0	9	1.7806	3.5370	198.635
5	E2	0	9	64.3197	25.4366	39.547
6	ETOH	0	9	57.3435	24.6788	43.037

AVERAGE PERCENTAGE OF FEMALES BY TREATMENT GROUP 1054

Obs	GROUP	_TYPE_	_FREQ_	MEAN	STD	CV
1	10	0	9	0.00000	0.00000	.
2	25	0	9	0.00000	0.00000	.
3	CONTR	0	9	0.00000	0.00000	.
4	DHT	0	9	0.00000	0.00000	.
5	E2	0	9	0.00000	0.00000	.
6	ETOH	0	9	1.38889	3.92837	282.843

AVERAGE LENGTH OF FROGS BY GROUP 1055

Obs	GROUP	_TYPE_	_FREQ_	MEAN	STD	CV
1	10	0	77	2.38551	0.20537	8.6091
2	25	0	48	2.53903	0.26360	10.3818
3	Control	0	58	2.51361	0.29859	11.8789
4	DHT	0	104	2.41714	0.24883	10.2945
5	E2	0	40	2.56458	0.32778	12.7810
6	ETOH	0	69	2.46785	0.27089	10.9767

AVERAGE WEIGHT OF FROGS BY GROUP 1056

Obs	GROUP	_TYPE_	_FREQ_	MEAN	STD	CV
1	10	0	77	1.48109	0.34933	23.5857
2	25	0	48	1.64568	0.40205	24.4305
3	Control	0	58	1.75386	0.54678	31.1760
4	DHT	0	104	1.50077	0.45081	30.0385
5	E2	0	40	1.63576	0.51305	31.3650
6	ETOH	0	69	1.56208	0.43097	27.5892

AVERAGE AGE OF FROGS IN DAYS AT END OF STUDY BY GROUP 1057

Obs	GROUP	_TYPE_	_FREQ_	MEAN	STD	CV
1	10	0	77	376.703	58.6103	15.5587
2	25	0	48	342.135	47.8134	13.9750
3	Control	0	58	336.750	66.0096	19.6019
4	DHT	0	104	368.533	68.7472	18.6543
5	E2	0	40	342.152	26.5496	7.7596
6	ETOH	0	69	359.681	65.3223	18.1612

ANALYSIS OF VARIANCE FOR LENGTH OF FROGS BETWEEN GROUPS 1058

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

The GLM Procedure

Class Level Information

Class	Levels	Values
GROUP	6	10 25 Control DHT E2 ETOH

Number of observations 153

NOTE: Due to missing values, only 135 observations can be used in this analysis.

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.90320512	0.18064102	2.47	0.0359
Error	129	9.44285747	0.07320045		
Corrected Total	134	10.34606259			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.087299	10.85520	0.270556	2.492407

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GROUP	5	0.90320512	0.18064102	2.47	0.0359

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GROUP	5	0.90320512	0.18064102	2.47	0.0359

ANALYSIS OF VARIANCE FOR LENGTH OF FROGS BETWEEN GROUPS 1060

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

The GLM Procedure

Class Level Information

Class	Levels	Values
GROUP	6	10 25 Control DHT E2 ETOH

Number of observations 194

NOTE: Due to missing values, only 162 observations can be used in this analysis.

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.43825553	0.08765111	1.37	0.2383
Error	156	9.97802868	0.06396172		
Corrected Total	161	10.41628421			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.042074	10.40884	0.252907	2.429728

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GROUP	5	0.43825553	0.08765111	1.37	0.2383

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GROUP	5	0.43825553	0.08765111	1.37	0.2383

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING LENGTH 1062

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

The UNIVARIATE Procedure
Variable: Resid

Moments

N	135	Sum Weights	135
Mean	0	Sum Observations	0
Std Deviation	0.26546014	Variance	0.07046909
Skewness	0.22001009	Kurtosis	-0.3647052
Uncorrected SS	9.44285747	Corrected SS	9.44285747
Coeff Variation	.	Std Error Mean	0.02284717

Basic Statistical Measures

Location		Variability	
Mean	0.00000	Std Deviation	0.26546
Median	-0.02028	Variance	0.07047
Mode	-0.06828	Range	1.36128
		Interquartile Range	0.37773

NOTE: The mode displayed is the smallest of 5 modes with a count of 3.

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	-2.5	Pr >= |M|	0.7308
Signed Rank	S	-117.5	Pr >= |S|	0.7974

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.989195	Pr < W	0.3771
Kolmogorov-Smirnov	D	0.053022	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.068716	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.452489	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	0.720692
99%	0.631720
95%	0.420692
90%	0.363100
75% Q3	0.198425
50% Median	-0.020280
25% Q1	-0.179308
10%	-0.354575
5%	-0.382900
1%	-0.533280
0% Min	-0.640591

Extreme Observations

------Lowest------		------Highest-----	
Value	Obs	Value	Obs
-0.640591	58	0.453425	28
-0.533280	84	0.531720	79
-0.467280	85	0.620692	136
-0.454900	120	0.631720	87
-0.388280	98	0.720692	135

Missing Values

Missing Value	Count	-----Percent Of----- All Obs	Missing Obs
.	18	11.76	100.00

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING LENGTH 1064

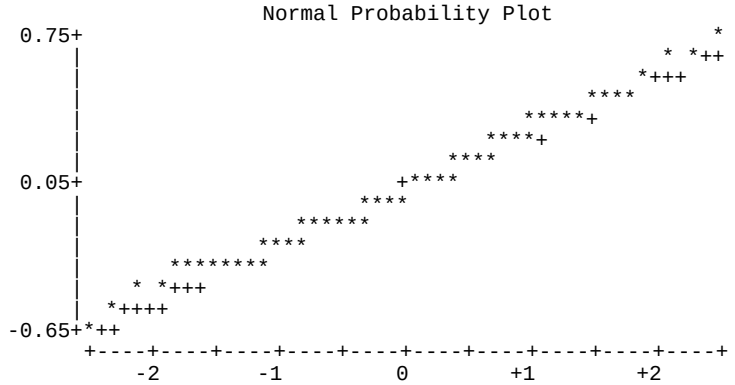
---------- SEX=F ----------

The UNIVARIATE Procedure
Variable: Resid

Stem	Leaf	#	Boxplot
7	2	1	
6	23	2	
5	3	1	
4	222235	6	
3	12222336777	11	
2	0022223457779	13	
1	02223355778899	14	
0	12222222245667777	17	
-0	988777766443332222	18	
-1	9988887777766654433110	22	
-2	88876555320	11	
-3	988887775544443	15	
-4	75	2	
-5	3	1	
-6	4	1	

Multiply Stem.Leaf by 10**-1

Normal Probability Plot



PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING LENGTH 1065

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

The UNIVARIATE Procedure
Variable: Resid

Moments

N	162	Sum Weights	162
Mean	0	Sum Observations	0
Std Deviation	0.24894846	Variance	0.06197533
Skewness	0.31544092	Kurtosis	0.99990473
Uncorrected SS	9.97802868	Corrected SS	9.97802868
Coeff Variation	.	Std Error Mean	0.01955924

Basic Statistical Measures

Location		Variability	
Mean	0.000000	Std Deviation	0.24895
Median	0.003363	Variance	0.06198
Mode	0.103363	Range	1.46290
		Interquartile Range	0.32346

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	2	Pr >= |M|	0.8138
Signed Rank	S	-12.5	Pr >= |S|	0.9834

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.979442	Pr < W	0.0164
Kolmogorov-Smirnov	D	0.037429	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.038106	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.389134	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	0.9033625
99%	0.8605833
95%	0.3669048
90%	0.3000417
75% Q3	0.1603625
50% Median	0.0033625
25% Q1	-0.1630952
10%	-0.3090952
5%	-0.3996375
1%	-0.5374167
0% Min	-0.5595333

Extreme Observations

------Lowest------		------Highest-----	
Value	Obs	Value	Obs
-0.559533	39	0.396042	22
-0.537417	155	0.403363	134
-0.533095	179	0.648363	121
-0.513222	63	0.860583	166
-0.489417	167	0.903363	101

Missing Values

Missing Value	Count	-----Percent Of----- All Obs	Missing Obs
.	32	16.49	100.00

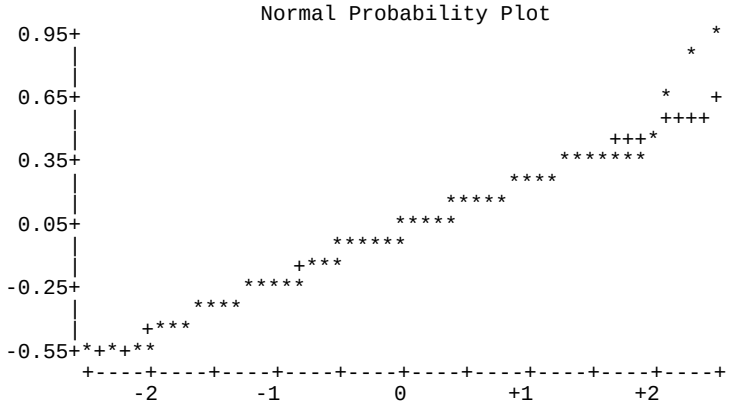
PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING LENGTH 1067

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

The UNIVARIATE Procedure
Variable: Resid

```
Stem Leaf                            #             Boxplot
   9 0                               1                0
   8 6                               1                0
   7
   6 5                               1                0
   5
   4 00                              2                |
   3 000002557778                   12                |
   2 012235555667788                15                |
   1 000000022233445566678889       25             +-----+
   0 000122223355556667788888999    26             *--+--*
  -0 99999877665444433222211        24             |     |
  -1 87655433221111000              17             +-----+
  -2 9887755554444332110            19                |
  -3 776543100                       9                |
  -4 972000                          6                |
  -5 6431                            4                |
     ----+----+----+----+----+-
Multiply Stem.Leaf by 10**-1
```

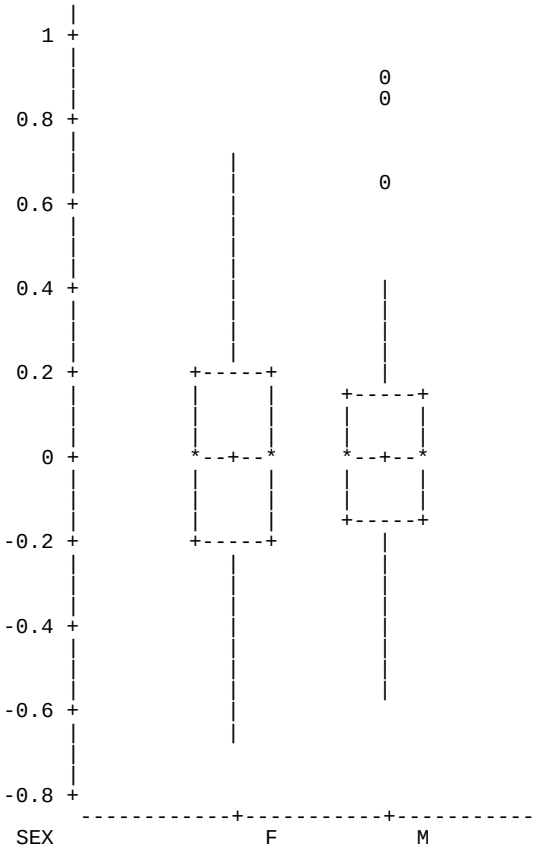
Normal Probability Plot



PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING LENGTH 1068

The UNIVARIATE Procedure
Variable: Resid

Schematic Plots



NONPARAMETRIC COMPARISON OF FROG LENGTH ACROSS GROUPS 1069

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

The NPAR1WAY Procedure

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	40	2141.00	2720.0	207.438789	53.525000
25	22	1699.50	1496.0	167.783300	77.250000
Control	25	1923.50	1700.0	176.467412	76.940000
DHT	2	178.50	136.0	54.883145	89.250000
E2	20	1549.00	1360.0	161.384599	77.450000
ETOH	26	1688.50	1768.0	179.142278	64.942308

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	9.9388
DF	5
Pr > Chi-Square	0.0770

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	40	14.0	19.851852	2.662548	0.350000
25	22	13.0	10.918519	2.153556	0.590909
Control	25	15.0	12.407407	2.265020	0.600000
DHT	2	1.0	0.992593	0.704444	0.500000
E2	20	11.0	9.925926	2.071427	0.550000
ETOH	26	13.0	12.903704	2.299353	0.500000

Average scores were used for ties.

Median One-Way Analysis

Chi-Square	5.4793
DF	5
Pr > Chi-Square	0.3602

NONPARAMETRIC COMPARISON OF FROG LENGTH ACROSS GROUPS 1071

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

The NPAR1WAY Procedure

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	24	1881.00	1956.00	211.993261	78.375000
25	15	1582.00	1222.50	172.974144	105.466667
Control	18	1456.00	1467.00	187.540207	80.888889
DHT	72	5359.50	5868.00	296.527103	74.437500
E2	12	1157.00	978.00	156.283506	96.416667
ETOH	21	1767.50	1711.50	200.445401	84.166667

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	6.9456
DF	5
Pr > Chi-Square	0.2247

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	24	12.0	12.00	2.267787	0.500000
25	15	11.0	7.50	1.850382	0.733333
Control	18	8.0	9.00	2.006202	0.444444
DHT	72	31.0	36.00	3.172083	0.430556
E2	12	9.0	6.00	1.671835	0.750000
ETOH	21	10.0	10.50	2.144254	0.476190

Average scores were used for ties.

Median One-Way Analysis

Chi-Square	7.8765
DF	5
Pr > Chi-Square	0.1632

ANALYSIS OF VARIANCE FOR WEIGHT OF FROGS BETWEEN GROUPS 1073

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

The GLM Procedure

Class Level Information

Class	Levels	Values
GROUP	6	10 25 Control DHT E2 ETOH

Number of observations 153

NOTE: Due to missing values, only 134 observations can be used in this analysis.

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2.71751620	0.54350324	2.51	0.0330
Error	128	27.66481514	0.21613137		
Corrected Total	133	30.38233134			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.089444	28.95896	0.464899	1.605373

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GROUP	5	2.71751620	0.54350324	2.51	0.0330

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GROUP	5	2.71751620	0.54350324	2.51	0.0330

ANALYSIS OF VARIANCE FOR WEIGHT OF FROGS BETWEEN GROUPS 1075

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

The GLM Procedure

Class Level Information

Class	Levels	Values
GROUP	6	10 25 Control DHT E2 ETOH

Number of observations 194

NOTE: Due to missing values, only 162 observations can be used in this analysis.

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.79520647	0.15904129	0.86	0.5087
Error	156	28.80682131	0.18465911		
Corrected Total	161	29.60202778			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.026863	27.92739	0.429720	1.538704

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GROUP	5	0.79520647	0.15904129	0.86	0.5087

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GROUP	5	0.79520647	0.15904129	0.86	0.5087

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING WEIGHT 1077

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

The UNIVARIATE Procedure
Variable: Resid

Moments

N	134	Sum Weights	134
Mean	0	Sum Observations	0
Std Deviation	0.45607689	Variance	0.20800613
Skewness	0.78265338	Kurtosis	0.6746575
Uncorrected SS	27.6648151	Corrected SS	27.6648151
Coeff Variation	.	Std Error Mean	0.03939904

Basic Statistical Measures

Location		Variability	
Mean	0.00000	Std Deviation	0.45608
Median	-0.07348	Variance	0.20801
Mode	0.30667	Range	2.42000
		Interquartile Range	0.60333

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	-12	Pr >= |M|	0.0465
Signed Rank	S	-382	Pr >= |S|	0.3983

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.962472	Pr < W	0.0010
Kolmogorov-Smirnov	D	0.092468	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.224995	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.348236	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	1.5448000
99%	1.2600000
95%	0.8948000
90%	0.5866667
75% Q3	0.2700000
50% Median	-0.0734848
25% Q1	-0.3333333
10%	-0.4933333
5%	-0.6261538
1%	-0.8336364
0% Min	-0.8752000

Extreme Observations

------Lowest------		------Highest-----	
Value	Obs	Value	Obs
-0.875200	84	0.993846	135
-0.833636	58	1.114800	79
-0.785200	98	1.206667	14
-0.765200	77	1.260000	124
-0.665200	88	1.544800	87

Missing Values

Missing Value	Count	-----Percent Of----- All Obs	Missing Obs
.	19	12.42	100.00

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING WEIGHT 1079

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

The UNIVARIATE Procedure
Variable: Resid

Stem	Leaf	#	Boxplot
15	4	1	0
14			
13			
12	16	2	0
11	1	1	|
10			|
9	69	2	|
8	79	2	|
7	13	2	|
6	68	2	|
5	13588899	8	|
4	014	3	|
3	111115777	9	|
2	124466678	9	+-----+
1	223369	6	| |
0	12456789	8	| + |
-0	9997774322111000	16	*-----*
-1	99765432211	11	| |
-2	977666554222100	15	| |
-3	887755433331	12	+-----+
-4	9999655432110	13	|
-5	733	3	|
-6	73322	5	|
-7	97	2	|
-8	83	2	|

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

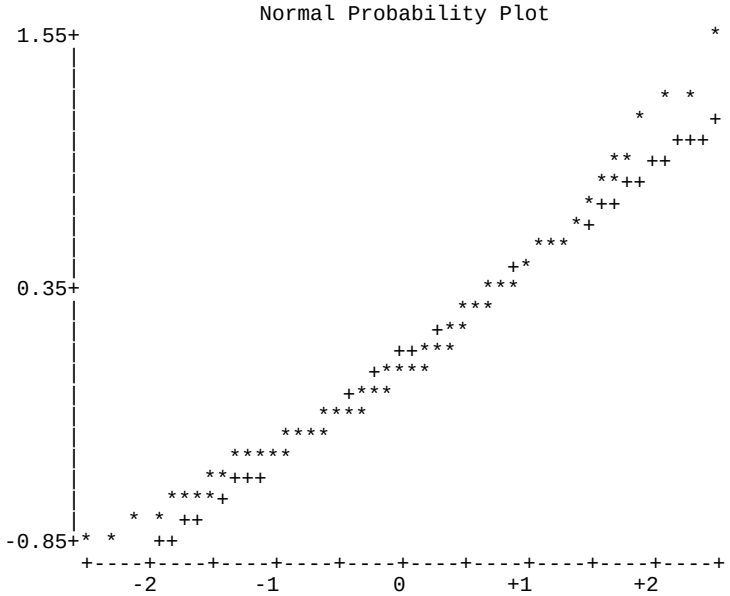
Multiply Stem.Leaf by 10**-1

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING WEIGHT 1080

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

The UNIVARIATE Procedure
Variable: Resid

Normal Probability Plot



PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING WEIGHT 1081

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

The UNIVARIATE Procedure
Variable: Resid

Moments

N	162	Sum Weights	162
Mean	0	Sum Observations	0
Std Deviation	0.42299451	Variance	0.17892436
Skewness	1.66369399	Kurtosis	5.77981391
Uncorrected SS	28.8068213	Corrected SS	28.8068213
Coeff Variation	.	Std Error Mean	0.03323359

Basic Statistical Measures

Location		Variability	
Mean	0.00000	Std Deviation	0.42299
Median	-0.03063	Variance	0.17892
Mode	-0.22875	Range	2.79958
		Interquartile Range	0.44792

NOTE: The mode displayed is the smallest of 2 modes with a count of 4.

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	-6	Pr >= |M|	0.3875
Signed Rank	S	-649.5	Pr >= |S|	0.2788

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.882296	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.095985	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.354957	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	2.887157	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	2.051250
99%	1.881250
95%	0.491250
90%	0.377333
75% Q3	0.211250
50% Median	-0.030625
25% Q1	-0.236667
10%	-0.462500
5%	-0.572667
1%	-0.744762
0% Min	-0.748333

Extreme Observations

------Lowest------		-----Highest-----	
Value	Obs	Value	Obs
-0.748333	167	1.20125	142
-0.744762	179	1.28750	22
-0.718750	108	1.40167	166
-0.688333	155	1.88125	121
-0.628750	147	2.05125	101

Missing Values

Missing Value	Count	-----Percent Of----- All Obs	Missing Obs
.	32	16.49	100.00

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING WEIGHT 1083

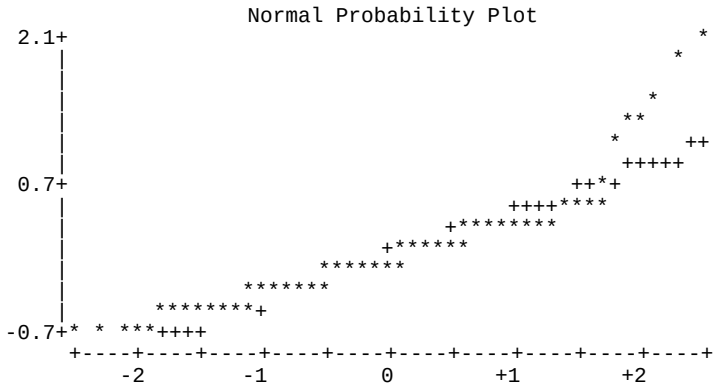
---------- SEX=M ----------

The UNIVARIATE Procedure
Variable: Resid

Stem	Leaf	#	Boxplot
20	5	1	*
18	8	1	*
16			
14	0	1	0
12	09	2	0
10	8	1	0
8			
6	1	1	|
4	0223996	7	|
2	000111233345556679001455577888	31	+-----+
0	012334455567899991122234456689	30	| + |
-0	9887765433222200099766655433222l0	33	*-----*
-2	977763220086665443333322221100	30	+-----+
-4	87755110866633210	17	|
-6	5429300	7	|

Multiply Stem.Leaf by 10**-1

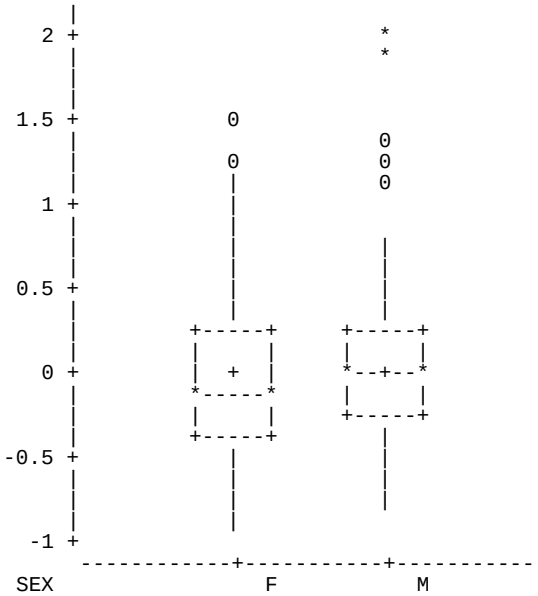
Normal Probability Plot



PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING WEIGHT 1084

The UNIVARIATE Procedure
Variable: Resid

Schematic Plots



NONPARAMETRIC COMPARISON OF FROG WEIGHT ACROSS GROUPS 1085

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

The NPAR1WAY Procedure

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	39	2190.50	2632.50	204.134406	56.166667
25	22	1584.00	1485.00	166.472482	72.000000
Control	25	2031.00	1687.50	175.067430	81.240000
DHT	2	195.00	135.00	54.490911	97.500000
E2	20	1388.00	1350.00	160.136203	69.400000
ETOH	26	1656.50	1755.00	177.713596	63.711538

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	8.2408
DF	5
Pr > Chi-Square	0.1435

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	39	12.0	19.50	2.638993	0.307692
25	22	12.0	11.00	2.152110	0.545455
Control	25	17.0	12.50	2.263223	0.680000
DHT	2	2.0	1.00	0.704443	1.000000
E2	20	11.0	10.00	2.070197	0.550000
ETOH	26	13.0	13.00	2.297432	0.500000

Average scores were used for ties.

Median One-Way Analysis

Chi-Square	11.3060
DF	5
Pr > Chi-Square	0.0456

NONPARAMETRIC COMPARISON OF FROG WEIGHT ACROSS GROUPS 1087

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

The NPAR1WAY Procedure

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	24	1944.50	1956.00	212.080099	81.020833
25	15	1551.50	1222.50	173.044998	103.433333
Control	18	1709.00	1467.00	187.617028	94.944444
DHT	72	5186.00	5868.00	296.648568	72.027778
E2	12	1105.00	978.00	156.347523	92.083333
ETOH	21	1707.00	1711.50	200.527508	81.285714

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	8.3092
DF	5
Pr > Chi-Square	0.1400

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

GROUP	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
10	24	13.0	12.00	2.267787	0.541667
25	15	10.0	7.50	1.850382	0.666667
Control	18	13.0	9.00	2.006202	0.722222
DHT	72	29.0	36.00	3.172083	0.402778
E2	12	7.0	6.00	1.671835	0.583333
ETOH	21	9.0	10.50	2.144254	0.428571

Average scores were used for ties.

Median One-Way Analysis

Chi-Square	8.8182
DF	5
Pr > Chi-Square	0.1165