Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

Data Requirement: :

     EPA DP Barcode D288775

     EPA MRID   458677-07
     EPA Guideline 70-1(Special Study)

Test material: **Purity:** 98.6%
Common name Atrazine
Chemical name: IUPAC
      CAS name 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
      CAS No. 1912-24-9
      Synonyms
      EPA PC Code: 80803

Primary Reviewer: Thomas M. Steeger, Ph.D., Senior Biologist   **Date:** April 6, 2003
Environmental Fate and Effects Division, ERB 4,
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EPA PC Code 080803

Date Evaluation Completed: 05/31/2003

CITATION: Goleman, W. L. and J. A. Carr. 2003. Response of larval *Xenopus laevis* to Atrazine
  Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology. The Institute of
  Environmental and Human Health, Texas Tech University, Texas Tech University Health Sciences Center,
  Lubbock, Texas. Sponsor: Syngenta Crop Protection, Inc., Laboratory Study ID ECORISK Number
  TTU-01.

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EXECUTIVE SUMMARY:

In a 78-day exposure, 48- to 72-hr post-hatch African clawed frog (*Xenopus laevis*) larvae were exposed to nominal concentrations of 1, 10 and 25 µg atrazine/L in FETAX medium, FETAX medium alone (negative control), 17-β estradiol, dihydrotestosterone, or solvent control (0.0025% ethanol) in a static renewal system where 50% exposure solution water changes occurred every 72 hours. For the first seven days, 60 - 65 larvae were maintained in 100 mL of exposure solution. On day 14, animals were transferred to 1 L of exposure solution, and by Day 21, animals were maintained in 4 L of exposure solution. At NF Stage 66 (forelimb emergence and complete tail resorption) animals were weighed, measured for snout-vent length and examined for gonadal gross morphology. Larynx and gonads also underwent histological analysis.

Mortality over the study period ranged from 10 to 14% for those animals that reached stage 66 by 80 days post-hatch. Time to complete metamorphosis was not significantly different across treatments although the specific time was not reported. In all treatments, weight and snout-vent length were inversely proportional to the number of days required to complete metamorphosis, *i.e.*, animals completing metamorphosis early tended to be larger than animals that took longer to complete metamorphosis. Sex ratios ranged from 48% to 50% male across all treatments except for estradiol, which skewed the ratio in favor of females (67%). While the incidence of intersex was correlated with atrazine treatment concentrations, only the 25 µg atrazine/L (4.7%) and estradiol-treated (7.4%) males had incidence rates significantly different from negative (0.6%) and solvent (0.0%) controls. Intersex in males treated with 25 µg atrazine/L contained distinguishable testicular and ovarian tissue, while males treated with estradiol sometimes contained ambiguous tissue structures.

There was no difference in the cross-sectional area of larynx dilator muscle in any atrazine-treated males relative to negative controls. Dihydrotestosterone-treated females had significantly larger cross-sectional dilator muscle areas than solvent control females.

Although the report concluded that atrazine did not impact length, weight, time to metamorphosis or dilator muscle area relative to controls, it did conclude that exposure to 25 µg atrazine/L appeared to significantly increase the number of intersex males and animals with discontinuous gonads. However, the observation that both body weight and length were inversely correlated with the length of time to complete metamorphosis suggested that animals in all treatment groups were developmentally impaired. Also, the fact that 17-β estradiol treatment only resulted in 67% females, further suggested that study animals were not entirely responsive to the positive control. Although dissolved oxygen did not appear to drop below 3.9 mg/L, ammonia levels ranged as high as 27 mg/L suggesting that the 50% static renewal and loading rates (number of tadpoles per liter of exposure solution) may have resulted in poor water quality that could, in turn, impair the development of test animals. Furthermore, roughly 42% of the animals were assumed not to have reached stage 66 by Day 78, suggesting that a large proportion of the animals were not developing at all. Because of the declining condition of the frogs with increased length of time to maturity, it is uncertain whether these animals completed metamorphosis and/or survived. Furthermore, because all the animals in the study had not undergone metamorphosis, the percent initiating metamorphosis, time to metamorphosis and percentage of gonadal abnormalities could not be accurately calculated relative to the total animals used in the study.

This study indicates that only one exposure level (25 ug/L) produced developmental effects in frogs, but because of design problems the study did not establish a dose-response relationship between atrazine exposure and developmental effects in frogs. The ability of this study to serve as a sensitive indicator of developmental effects is also in question because of the impaired condition of the animals.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Nonguideline Study

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

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

A. MATERIALS:

1. Test Material   Atrazine

Description: Not reported

Lot No./Batch No. :   Not reported

Purity: 98.6%

Stability of compound under test conditions: Not reported

Storage conditions of test chemicals: Not reported

2. Test organism:

Species: African clawed frog (*Xenopus laevis*)

.

Age at test initiation: 48 - 72 hr larvae
Weight at study initiation: (mean and range) Not reported
Length at study initiation: (mean and range) Not reported

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Source: Six breeding pairs of *X. laevis* obtained from Xenopus Express (Homosassa, FL) artificially induced to spawn.

B. STUDY DESIGN:

Objective: 1. To determine the response of larval *Xenopus laevis* to atrazine by assessing the metamorphosis and reproductive indices when animals are exposed from 48 - 72 hours after hatching until the completion of metamorphosis. Indices to be evaluated include percent initiating metamorphosis, percent completing metamorphosis, time to metamorphosis, percentage of intersex gonads, fresh post-mortem body weight, snout-vent length, and laryngeal size.

1. Experimental Conditions

a) **Range-finding Study**: Trials 1 - 3 were conducted in 0.1x Holtfreter's solution and were terminated due to unexpectedly high mortality in all concentrations. In Trial 1, larvae were transferred 48 hr post-hatch and all the transferred animals died within 24 hours of transfer from "damaged abdomens". In Trials 2 and 3, exposures began when larvae were less than 24 hrs old resulting in "unacceptably high mortality" and poor growth leaving study authors to conclude that 0.1x Holtfretter's was unsuitable as a medium for raising larvae. FETAX medium was used in the fourth and final attempt at a definitive study.

b) **Definitive Study**

Table 1 . Experimental Parameters

Parameter	Details
Acclimation: period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	breeding adults maintained separately for 7 days before breeding; maintained in 45-L glass tanks containing 18-L of ultra-pure water at $22 \pm 1^{\circ}C$. Beginning 4 days prior to breeding, adult tank water changed to FETAX by daily 50% medium changes.
Duration of the test	78 days or until frogs reached NF stage 66 (forelimb emergence and complete tail resorption) of development
Test condition static/flow- through Type of dilution system for flow-through method. Renewal rate for static renewal	static renewal NA 50% exposure solution change every 72 hours

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Parameter	Details
Aeration, if any	not reported
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass beaker Initially 250 mL; on Day 7 transferred to 2-L beakers; on Day 21 transferred to 10-L beakers Initially 100 mL; on Day 7 filled with 1-L; on Day 21 filled with 4 L
Source of dilution water Quality:	tap water run through reverse osmosis and deionizer to convert to ultra-pure water
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Ammonia Nitrite Metals Pesticides Chlorine Temperature Salinity Intervals of water quality measurement	 not reported range: 6.9 to 8.3 range: 3.9 to 9.3 mg/L; mean 6.7 mg/L not reported not reported range: 0 to 27.1 mg/L (unionized 0 - 0.24 mg/L); mean 9.4 mg/L not reported not reported not reported not reported range: 19 to 22.8ºC range: 0.6 to 1.2 ppt temperature monitored daily; pH, conductivity, salinity, DO and ammonia monitored on freshly prepared stock solutions and 72-hr exposure solutions (every 72 hours).
Number of replicates/groups: negative control: water treated ones:	11 replicates for atrazine and negative control; 6 replicates for ethanol, dihydrotestosterone and 17-β estradiol (0.1 µg/mL) positive controls.

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Parameter	Details
Number of organisms per replicate /groups:	60 - 65 larvae per replicate
Biomass loading rate	Initially, 650/L, at Day 7 65/L and at Day 21 onward 16.3/ L
Test concentrations: nominal: measured:	0, 1, 10 and 25 µg/L 1.07 ± 0.02 µg/L, 10.3 ± 0.15 µg/L, 19.5 ± 0.21 µg/L
Solvent (type, percentage, if used)	FETAX solution for atrazine treated and negative control steroids and solvent control in 0.0025% ethanol/FETAX. FETAX medium: NaCl 0.625 g/L; $NaHCO_3$ 0.096 g/L; KCl 0.03 g/L; $CaCl_2$ 0.015 g/L, $CaSO_4$-$2H_2O$ 0.06 g/L; $MgSO_4$ 0.075 g/L
Lighting	12 hrs light, 12 hrs dark
Feeding	not reported
Recovery of chemical Level of Quantitation Level of Detection	Measured using ELISA (RAPID Strategic Diagnostics, Newark, DE); approximately 10% of samples verified by second analysis.
Positive control {if used, indicate the chemical and concentrations}	dihydrotestosterone (0.1 µg/L in 0.0025% ethanol) 17-β estradiol (0.1 µg/L in 0.0025% ethanol)
Other parameters, if any	

2. Observations:

Table 2: Observations

Criteria	Details
Parameters measured including the sublethal effects/toxicity symptoms	water quality (excess food, etc.); % mortality, % showing forelimb emergence, % metamorphosed (complete tail resorption)

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Observation intervals	monitored daily for changes in general health and for parameters described above.
Were raw data included?	
Other observations, if any	

II. RESULTS and DISCUSSION:

Atrazine concentrations in both the freshly prepared stock solutions and the exposure tanks were relatively consistent with nominal values (**Table 3**).

Table 3. Nominal and mean-measured atrazine concentrations in freshly prepared stock solutions and exposure tanks.

Nominal	Stock Solution	Exposure Tank
1 µg/L	1.02 ± 0.03 µg/L	1.07 ± 0.02 µg/L
10 µg/L	9.99 ± 0.29 µg/L	10.3 ± 0.15 µg/L
25 µg/L	21.3 ± 0.67 µg/L	19.5 ± 0.21 µg/L

Estradiol concentrations in exposure tanks A through F ranged from 5,520 pg/mL to 11,700 pg/mL (mean: 9,255 pg/mL) on August 21. On October 5, 2001, estradiol levels ranged from 4,764 to 17,467 pg/mL (mean: 9,897 pg/mL). Therefore, average estradiol concentrations were 0.009 µg/L and were roughly 9% of the desired nominal estradiol concentration of 0.1%.

Post-hatch mortality across all study groups ranged from 10 to 14% (**Table 4**). There was no difference in post-hatch mortality for atrazine treatments and the negative control (ANOVA, $p = 0.2$) or for hormone treatments and the ethanol control (ANOVA, $p = 0.7$). There was no difference in body weight or snout-vent length among atrazine-treated and negative controls. Time to complete metamorphosis (NF stage 66) varied inversely with body weight and SVL; those animals reaching NF-stage 66 first in each tank were significantly larger than those animals that were the last, *i.e.*, animals reaching NF-stage 66 by Day 78, in every treatment (paired t-test, $p<0.05$). Based on the report figure depicting body weights, the average weight of animals reaching stage 66 first ranged from 0.48 to 0.62 g, while the last animals to reach stage 66 had body weights ranging from 0.26 to 0.30 g. Average snout-vent length for animals reaching stage 66 first ranged from 16 to 17 mm while lengths for animals reaching stage 66 last ranged from 13 to 13.5 mm roughly. Body weight and SVL were also inversely correlated with time to complete metamorphosis in hormone-treated and solvent controls. Body weights for animals reaching stage 66 first in hormone and solvent-treated animals ranged from 0.48 to 0.54 g, while late stage 66 animals ranged from 0.24 to 0.25 g. Estradiol-treated females had significantly longer SVL than solvent controls (ANOVA, $p = 0.02$).

The percentage of animals reaching complete tail resorption varied between 37 - 52% during the 78-day period. Fewer atrazine-treated (referred to as a "weak trend" by authors) animals reached (ANOVA trend test, $p =$

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0.03) and completed tail resorption (ANOVA trend test, p = 0.04). However, the general ANOVA F-test revealed no significant effect of atrazine on either parameter compared to negative controls.

The incidence of gross gonadal deformities was less than 5% in all treatments. The incidence of edema was correlated with atrazine concentration (Cochran-Armitage trend test, Z = -2.3, p=0.02); however, a chi-square homogeneity test revealed that none of the atrazine concentrations significantly increased the incidence of edema compared to negative controls (p = 0.1). Abnormal swimming was also correlated with atrazine concentration (Cochran-Armitage trend test, Z = -2.90, p = 0.004), but only larvae exposed to 25 µg atrazine/L exhibited significantly greater incidence of abnormal swimming (chi-square homogeneity test, p = 0.04).

There were no detectable effects on sex ratio with ratios ranging from 48 to 50% males in all treatments except the 25 µg atrazine/L treatment. There was a slight reduction in percentage (45%) of males in the 25 µg atrazine/L. Neither ethanol nor dihyrotestosterone significantly affected sex ratios. Estradiol significantly reduced the percentage of males to 26% and increased the percentage of females to 67%. Approximately 7% of the estradiol-treated animals were classified as intersex based on gross morphology.

Exposure to either 25 µg atrazine/L or estradiol significantly increased the percentage of individuals with intersex gonads. Although the incidence of intersex increased with increasing atrazine concentrations (Cochran-Armitage, Z= 3.6, p = 0.0003), only 25 µg atrazine/L significantly increased the average incidence of intersex animals (4.7%) per tank compared to negative control (0.6%) (p = 0.0061) (**Table 5**). There was also a significant correlation between the incidence of discontinuous gonads and atrazine concentration (Cochran-Armitage Z = 2.9, p = 0.0042) although 25 µg atrazine/L was the only concentration to increase the incidence of discontinuous gonads compared to negative controls. The percentage of intersex gonads was also significantly higher in the estradiol-treated group compared to the solvent control. (Kruskall-Wallis, p = 0.01).

Table 5. Total number of frogs identified as intersex by treatment.

Treatment	Total Animals	Intersex	Percentage
0	334	2	0.6%
1 µg atrazine/L	309	3	0.97%
10 µg atrazine/L	276	1	0.36%
25 µg atrazine/L	296	14	4.7%
estradiol	135	10	7.4%
solvent control	160	0	0.0%

Intersex animals in the 25 µg atrazine/L group tended to have obvious testicular or ovarian tissues, while estradiol-treated animals revealed "ambiguous" gonadal tissue in some cases.

There were no significant differences in male dilator muscle size within any of the negative control or atrazine treatments (**Table 6**). Total muscle cross-sectional area was 20 - 25% larger in males than females in all atrazine-treated groups. There were no significant effects of atrazine on total cross-sectional area in females (p = 0.5). Exposure to dihydrotestosterone increased total muscle cross-sectional area approximately 2-fold

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in both males ($p > 0.0001$) and females ($p < 0.0001$). Total muscle cross-sectional area was larger in males than in females in the solvent control group but not in the estradiol and DHT-treated groups.

Table 6. Mean ($\pm$ standard error) of total dilator muscle cross-sectional area (mm^2) in Nieuwkoop Faber stage 66 *Xenopus laevis* exposed to atrazine or sex steroids[a].

Treatment	Males	Females
negative FETAX control b	0.154 ± 0.004 (n = 45)	0.117 ± 0.006 (n = 11)
1 µg atrazine/L	0.167 ± 0.005 (n = 41)	0.118 ± 0.006 (n = 10)
10 µg atrazine/L	0.168 ± 0.004 (n = 44)	0.129 ± 0.007 (n = 11)
25 µg atrazine/L	0.168 ± 0.004 (n = 42)	0.122 ± 0.006 (n = 12)
solvent control (0.0025% ethanol)	0.161 ± 0.008 (n = 6)	0.121 ± 0.013 (n = 6)
dihydrotestosterone	0.301 ± 0.012[*] (n = 6)	0.313 ± 0.024[*] (n = 6)
17-β estradiol	0.148 ± 0.010 (n = 6)	0.141 ± 0.009 (n = 6)

[a] Combined largest cross-sectional area through the right and left laryngeal dilator muscles.
[b] FETAX: frog embryo teratogenesis assay– *Xenopus*.
[*] significantly grater than ethanol control (p <0.0001)

F. REVIEWER'S COMMENTS:

 For several days, breeding adults were apparently maintained in ultra-pure water before transitioning them into FETAX.

Although samples were collected at the beginning, middle and end of the study for steroid analyses, the report also states though that estradiol was measured using RIA kit (Diagnostic Products Corporation, Los Angeles, CA; LOD 20 pg/mL). DHT values, though, are reported as nominal, and it is unclear whether any effort was made to measure DHT. Measured estradiol concentrations were 0.009 µg/L and were roughly 9% of nominal. Coefficients of variation for the means ranged from 46.5 to 68.9%.

Water samples were collected of freshly prepared stock solutions and of exposure solutions just prior to renewal.

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Although the study starts with 60 to 65 larvae per replicate with 11 replicates in the atrazine-treated groups, the results are expressed in terms of 20 - 30 larvae per replicate. If mortality ranged from 10 to 14%, it would leave roughly 52 larvae per replicate, and 42% of the animals would be unaccounted.. It is assumed that the remaining animals failed to reach metamorphosis by Day 78.

The feeding regime was not discussed; however, the report states that frog brittle feed was analyzed by immunoassay and "yielded inconclusive results". Because immunoassays are specific to the antigen of interest, it is presumed that the immunoassay was for atrazine and that something, presumably atrazine, gave variable results, *i.e.*, "yielded inconclusive results." The report states that since a GC/MS method for analyzing frog brittle has not been developed, no data on the food were available (reported).

Body weights of metamorphs were negatively correlated with the length of time to metamorphosis; the number of intersex animals was positively correlated with atrazine concentration.

Although not discussed in the results section, the number of discontinuous gonads appeared to be positively correlated with atrazine treatment. Extrapolating from the figure depicting the percentage of discontinuous gonads there appeared to be 3.5% in controls, and 4%, 7% and 8% in 1, 10 and 25 µg atrazine/L. The number of discontinuous gonads was significantly higher than controls in the 25 µg atrazine/L treatment (chi-square, $p < 0.05$)

The study concluded that estradiol treatment reduced the number of phenotypic males to 25%; however, previous literature suggested that estradiol treatment should result in 100% females.

Body weights and time to metamorphosis were not reported in the study nor was the feeding regime mentioned. Body weights and lengths were extrapolated from figures.

Although the report concluded that atrazine did not impact length, weight, time to metamorphosis or dilator muscle area relative to controls, it did conclude that exposure to 25 µg atrazine/L appeared to significantly increase the number of intersex males and animals with discontinuous gonads. However, the observation that both body weight and length were inversely correlated with the length of time to complete metamorphosis suggested that animals in all treatment groups were developmentally impaired. In addition, 17-β estradiol treatment only resulted in 67% females, which further suggested that study animals were not entirely responsive to the positive control. Although dissolved oxygen did not appear to drop below 3.9 mg/L, ammonia levels did range as high as 27 mg/L, suggesting that the 50% static renewal and loading rates (number of tadpoles per liter of exposure solution) may have resulted in poor water quality, which in turn, accounted for the slow development of test animals.

Roughly 42% of the animals were assumed not to have reached stage 66 by Day 78, suggesting that a large proportion of the animals were not developing at all. Given the declining condition of the frogs with increased length of time to maturity, it is uncertain whether these animals would have undergone metamorphosis and/or survived. Furthermore, since all the animals in the study had not undergone metamorphosis, the percent initiating metamorphosis, time to metamorphosis and percentage of gonadal abnormalities could not be accurately calculated relative to the total animals used in the study.

After 80 days, only 49-61% of the organisms reached forelimb emergence (stage 58) and only 37-52% completed metamorphosis (stage 66). According to Nieuwkoop and Faber, the nominal days to stages 58 and

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66 are approximately 44 and 58 days, respectively. Although this can vary between laboratories and conditions, the developmental rates observed in this study are exceedingly slow. (At the National Health and Environmental Effects Research Laboratory researchers typically observe nearly 100% tail resorption in approximately 50-54 days.) Furthermore, there is considerable divergence in development because at least 51-39% of the tested organisms are at or below stage 57, while some have completed metamorphosis.

Taken together, these observations suggest that the organisms were in poor condition. It is unclear, though, how such retarded development could affect sexual differentiation.

The report states that there is no evidence that androgens affected gonadal development in *X. laevis*. While it is apparently true that testosterone is incapable of causing masculinization, DHT, the positive androgen control used in this study, has been shown to masculinize *X. laevis*.

Because only about 50% of the tested organisms reached stage 66, and all of the analyses were conducted on stage 66 organisms, the sampling strategy was probably biased and did not represent the population in the test.

The percentage of tadpoles with abnormal swimming was positively correlated (r=0.41; p=0.006) with atrazine concentration (see attached SAS$^©$ [Statistical Analysis System, Release 8.01, Cary, North Carolina] output); the percentage of tadpoles with edema was also positively correlated (r= 0.40; p=0.007) with atrazine concentration.

The ability of this study to serve as a sensitive indicator of developmental effects is in question given the seemingly impaired condition of the animals.

G. **CONCLUSIONS:**   Although the report concluded that atrazine did not impact length, weight, time to metamorphosis or dilator muscle area relative to controls, it did conclude that exposure to 25 µg atrazine/L appeared to significantly increase the number of intersex males and animals with discontinuous gonads. However, the observation that both body weight and length were inversely correlated with the length of time to complete metamorphosis suggested that animals in all treatment groups were developmentally impaired. Also, the fact that 17-β estradiol treatment only resulted in 67% females, further suggested that study animals were not entirely responsive to the positive control. Although dissolved oxygen did not appear to drop below 3.9 mg/L, ammonia levels ranged as high as 27 mg/L suggesting that the 50% static renewal and loading rates (number of tadpoles per liter of exposure solution) may have resulted in poor water quality that could, in turn, impair the development of test animals. Furthermore, roughly 42% of the animals were assumed not to have reached stage 66 by Day 78, suggesting that a large proportion of the animals were not developing at all. Because of the declining condition of the frogs with increased length of time to maturity, it is uncertain whether these animals completed metamorphosis and/or survived. Furthermore, because all the animals in the study had not undergone metamorphosis, the percent initiating metamorphosis, time to metamorphosis and percentage of gonadal abnormalities could not be accurately calculated relative to the total animals used in the study.

This study indicates that only one exposure level (25 ug/L) produced developmental effects in frogs, but because of design problems the study did not establish a dose-response relationship between atrazine exposure and developmental effects in frogs. The ability of this study to serve as a sensitive indicator of developmental effects is also in question because of the impaired condition of the animals.

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H. REFERENCES:

Nieuwkoop, P. D. and J. Faber. 1967. Normal table of *Xenopus laevis* (Daudin). North-Holland Publishing Company, Amsterdam.

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Table 4. Hatching, mortality, metamorphosis, and gross developmental abnormalities in *X. laevis* tadpoles exposed to atrazine or steroid hormones[a].

Treatment	Hatching (%)	Mortality[b] (%)	Forelimb Emergence (%)	Tail Resorption (%)	Bent Tails (%)	Edema (%)	Abnormal Swimming (%)
Negative	94.9 ± 1.02	10.4 ± 1.07	60.8 ± 3.00	51.5 ± 2.91	0.94 ± 0.43	0.15 ± 0.15	1.37 ± 0.44
1 µg/L	93.6 ± 1.12	11.4 ± 1.20	59.0 ± 3.31	48.4 ± 3.00	0.49 ± 0.36	0.30 ± 0.30	2.15 ± 0.53
10 µg/L	94.5 ± 0.093	14.1 ± 0.99	52.2 ± 3.85	42.3 ± 3.42	2.13 ± 0.46	0.44 ± 0.23	3.12 ± 0.68
25 µg/L	96.0 ± 0.82	12.4 ± 1.58	52.5 ± 2.39	44.4 ± 2.14	1.22 ± 0.33	1.04 ± 0.33	3.75 ± 0.64*
ethanol (0.0025%)	93.7 ± 1.47	11.7 ± 1.62	53.8 ± 4.93	46.1 ± 3.84	0.84 ± 0.56	0.57 ± 0.36	1.99 ± 0.73
Dihydrotestosterone	94.6 ± 2.32	12.2 ± 2.84	48.8 ± 5.36	37.2 ± 4.86	0.80 ± 0.36	1.38 ± 0.49	2.98 ± 0.94
17-β estradiol	92.9 ± 2.21	15.2 ± 4.16	51.2 ± 1.91	42.8 ± 2.28	0.82 ± 0.37	0.57 ± 0.36	1.69 ± 0.60

[a] Values are the mean ± standard error of eleven (FETAX medium) or six (ethanol co-solvent) replicates. Sample size per replicate ranged from 20 - 30 animals.
[b] 80-day post-hatch mortality.
* significantly different from FETAX medium (negative) control

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              PERCENT SURVIVAL BY TREATMENT GROUP                           236

    Obs     TREAT    _TYPE_    _FREQ_    SURVIVE     STD        CV

     1      0.0        0         11      89.5720    3.5310     3.9420
     2      1.0        0         11      88.5690    3.9837     4.4979
     3      10         0         11      85.9008    3.2759     3.8136
     4      25         0         11      87.6466    5.2333     5.9709
     5      DHT        0          6      87.8023    6.9606     7.9276
     6      ETOH       0          6      88.2824    3.9624     4.4884
     7      Estr       0          6      84.8416   10.1787    11.9972


            PERCENT OF EGGS HATCHED BY TREATMENT GROUP                      237

    Obs     TREAT    _TYPE_    _FREQ_    HATCHED     STD        CV

     1      0.0        0         11      94.9294    3.38528    3.56610
     2      1.0        0         11      93.6032    3.70056    3.95346
     3      10         0         11      94.5459    3.09986    3.27868
     4      25         0         11      95.9701    2.71563    2.82966
     5      DHT        0          6      94.5905    5.68238    6.00734
     6      ETOH       0          6      93.6631    3.59493    3.83815
     7      Estr       0          6      92.8788    5.41676    5.83207


        PERCENT OF TADPOLES WITH BENT TAILS BY TREATMENT GROUP             238

    Obs     TREAT    _TYPE_    _FREQ_     BENT       STD        CV

     1      0.0        0         11      0.93716    1.41694   151.195
     2      1.0        0         11      0.48822    1.17785   241.256
     3      10         0         11      2.12556    1.52127    71.571
     4      25         0         11      1.21942    1.11046    91.064
     5      DHT        0          6      0.80232    0.87911   109.570
     6      ETOH       0          6      0.83525    1.37177   164.234
     7      Estr       0          6      0.82068    0.90009   109.676


          PERCENT OF TADPOLES WITH EDEMA BY TREATMENT GROUP               239

    Obs     TREAT    _TYPE_    _FREQ_    EDEMA       STD        CV

     1      0.0        0         11      0.14903    0.49428   331.662
     2      1.0        0         11      0.30311    0.67450   222.524
     3      10         0         11      0.43770    0.74986   171.316
     4      25         0         11      1.03520    1.08659   104.965
     5      DHT        0          6      1.37917    1.21355    87.992
     6      ETOH       0          6      0.56644    0.87922   155.219
     7      Estr       0          6      0.56984    0.88284   154.928

    PERCENT OF TADPOLES WITH ABNORMAL SWIMMING BY TREATMENT GROUP         240

    Obs     TREAT    _TYPE_    _FREQ_    SWIM        STD        CV

     1      0.0        0         11      1.37132    1.46806   107.055
     2      1.0        0         11      2.15034    1.76982    82.304
     3      10         0         11      3.11772    2.25729    72.402
     4      25         0         11      3.75251    2.13240    56.826
     5      DHT        0          6      2.98382    2.30207    77.152
     6      ETOH       0          6      1.98706    1.77746    89.452
     7      Estr       0          6      1.69367    1.46398    86.438


            PERCENT METAMORPHOSIS BY TREATMENT GROUP                       241

    Obs     TREAT    _TYPE_    _FREQ_    METAMORP    STD        CV

     1      0.0        0         11      51.5133    9.6405    18.7146
     2      1.0        0         11      48.3625    9.9718    20.6188
     3      10         0         11      42.2644   11.3256    26.7971
     4      25         0         11      44.3954    7.1060    16.0061
     5      DHT        0          6      37.1615   11.8957    32.0109
     6      ETOH       0          6      46.0801    9.4053    20.4107
     7      Estr       0          6      42.7528    5.5845    13.0622
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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```
                           The NPAR1WAY Procedure

                  Wilcoxon Scores (Rank Sums) for Variable PMET
                        Classified by Variable TREAT

                        Sum of      Expected      Std Dev         Mean
          TREAT    N     Scores     Under H0      Under H0        Score
          âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
          0.0     11     459.00      346.50       54.267424    41.727273
          1.0     11     423.50      346.50       54.267424    38.500000
          10      11     298.00      346.50       54.267424    27.090909
          25      11     312.00      346.50       54.267424    28.363636
          DHT      6     114.50      189.00       41.997885    19.083333
          ETOH     6     199.00      189.00       41.997885    33.166667
          Estr     6     147.00      189.00       41.997885    24.500000
                    Average scores were used for ties.
                          Kruskal-Wallis Test

                      Chi-Square         9.9774
                      DF                      6
                      Pr > Chi-Square    0.1256


          Median Scores (Number of Points Above Median) for Variable PMET
                        Classified by Variable TREAT

                        Sum of      Expected      Std Dev         Mean
          TREAT    N     Scores     Under H0      Under H0        Score
          âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
          0.0     11      8.0         5.50        1.516305     0.727273
          1.0     11      7.0         5.50        1.516305     0.636364
          10      11      5.0         5.50        1.516305     0.454545
          25      11      4.0         5.50        1.516305     0.363636
          DHT      6      2.0         3.00        1.173477     0.333333
          ETOH     6      3.0         3.00        1.173477     0.500000
          Estr     6      2.0         3.00        1.173477     0.333333


                    Average scores were used for ties.



                          Median One-Way Analysis

                      Chi-Square         5.2473
                      DF                      6
                      Pr > Chi-Square    0.5125
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable SUR
Classified by Variable TREAT

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0.0	11	426.50	346.50	54.251705	38.772727
1.0	11	377.00	346.50	54.251705	34.272727
10	11	240.50	346.50	54.251705	21.863636
25	11	328.50	346.50	54.251705	29.863636
DHT	6	208.00	189.00	41.985719	34.666667
ETOH	6	185.50	189.00	41.985719	30.916667
Estr	6	187.00	189.00	41.985719	31.166667

Average scores were used for ties.
Kruskal-Wallis Test

Chi-Square   5.4728
DF   6
Pr > Chi-Square   0.4848

Median Scores (Number of Points Above Median) for Variable SUR
Classified by Variable TREAT

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0.0	11	7.750	5.50	1.479165	0.704545
1.0	11	6.750	5.50	1.479165	0.613636
10	11	2.750	5.50	1.479165	0.250000
25	11	4.750	5.50	1.479165	0.431818
DHT	6	4.000	3.00	1.144735	0.666667
ETOH	6	2.000	3.00	1.144735	0.333333
Estr	6	3.000	3.00	1.144735	0.500000

Average scores were used for ties.

Median One-Way Analysis

Chi-Square   6.9240
DF   6
Pr > Chi-Square   0.3279

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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NONPARAMETRIC COMPARISON OF PERCENT OF TADPOLES WITH ABNORMAL SWIMMING ACROSS TREATMENTS 246

                        The NPAR1WAY Procedure

              Wilcoxon Scores (Rank Sums) for Variable SWIM
                     Classified by Variable TREAT

                        Sum of      Expected      Std Dev        Mean
        TREAT      N     Scores      Under H0      Under H0       Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0       11     243.50       346.50      53.920541    22.136364
        1.0       11     321.00       346.50      53.920541    29.181818
        10        11     390.00       346.50      53.920541    35.454545
        25        11     462.50       346.50      53.920541    42.045455
        DHT        6     221.50       189.00      41.729430    36.916667
        ETOH       6     150.50       189.00      41.729430    25.083333
        Estr       6     164.00       189.00      41.729430    27.333333


                    Average scores were used for ties.


                          Kruskal-Wallis Test
                     Chi-Square           9.1688
                     DF                        6
                     Pr > Chi-Square      0.1643

          Median Scores (Number of Points Above Median) for Variable SWIM
                     Classified by Variable TREAT

                        Sum of      Expected      Std Dev        Mean
        TREAT      N     Scores      Under H0      Under H0       Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0       11       4.0         5.50       1.516305     0.363636
        1.0       11       5.0         5.50       1.516305     0.454545
        10        11       5.0         5.50       1.516305     0.454545
        25        11       9.0         5.50       1.516305     0.818182
        DHT        6       4.0         3.00       1.173477     0.666667
        ETOH       6       1.0         3.00       1.173477     0.166667
        Estr       6       3.0         3.00       1.173477     0.500000


                    Average scores were used for ties.



                         Median One-Way Analysis

                     Chi-Square           8.6461
                     DF                        6
                     Pr > Chi-Square      0.1945
NONPARAMETRIC COMPARISON OF PERCENT OF TADPOLES WITH BENT TAILS ACROSS TREATMENTS     248

                        The NPAR1WAY Procedure

              Wilcoxon Scores (Rank Sums) for Variable BENT
                     Classified by Variable TREAT

                        Sum of      Expected      Std Dev        Mean
        TREAT      N     Scores      Under H0      Under H0       Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0       11     323.50       346.50      50.384440    29.409091
        1.0       11     254.00       346.50      50.384440    23.090909
        10        11     491.50       346.50      50.384440    44.681818
        25        11     374.00       346.50      50.384440    34.000000
        DHT        6     161.00       189.00      38.992820    26.833333
        ETOH       6     174.00       189.00      38.992820    29.000000
        Estr       6     175.00       189.00      38.992820    29.166667
                    Average scores were used for ties.

                          Kruskal-Wallis Test

                     Chi-Square          10.7175
                     DF                        6
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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```
                            Pr > Chi-Square     0.0975

          Median Scores (Number of Points Above Median) for Variable BENT
                        Classified by Variable TREAT

                          Sum of      Expected      Std Dev        Mean
        TREAT      N       Scores     Under H0      Under H0       Score
        ââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0       11      4.218750       5.50      1.468156      0.383523
        1.0       11      2.281250       5.50      1.468156      0.207386
        10        11      9.062500       5.50      1.468156      0.823864
        25        11      7.125000       5.50      1.468156      0.647727
        DHT        6      3.093750       3.00      1.136215      0.515625
        ETOH       6      2.125000       3.00      1.136215      0.354167
        Estr       6      3.093750       3.00      1.136215      0.515625


                      Average scores were used for ties.



                          Median One-Way Analysis

                      Chi-Square          10.9792
                      DF                        6
                      Pr > Chi-Square      0.0890
       NONPARAMETRIC COMPARISON OF PERCENT OF TADPOLES WITH EDEMA ACROSS TREATMENTS      250

                          The NPAR1WAY Procedure


                 Wilcoxon Scores (Rank Sums) for Variable EDEMA
                        Classified by Variable TREAT

                          Sum of      Expected      Std Dev        Mean
        TREAT      N       Scores     Under H0      Under H0       Score
        ââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0       11       266.50      346.50      45.035250     24.227273
        1.0       11       300.00      346.50      45.035250     27.272727
        10        11       314.50      346.50      45.035250     28.590909
        25        11       417.00      346.50      45.035250     37.909091
        DHT        6       264.50      189.00      34.853050     44.083333
        ETOH       6       192.50      189.00      34.853050     32.083333
        Estr       6       198.00      189.00      34.853050     33.000000
                      Average scores were used for ties.


                          Kruskal-Wallis Test

                      Chi-Square          10.2116
                      DF                        6
                      Pr > Chi-Square      0.1160
          Median Scores (Number of Points Above Median) for Variable EDEMA
                        Classified by Variable TREAT

                          Sum of      Expected      Std Dev        Mean
        TREAT      N       Scores     Under H0      Under H0       Score
        ââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0       11      3.619048       5.50      1.046350      0.329004
        1.0       11      4.357143       5.50      1.046350      0.396104
        10        11      5.095238       5.50      1.046350      0.463203
        25        11      7.309524       5.50      1.046350      0.664502
        DHT        6      4.523810       3.00      0.809776      0.753968
        ETOH       6      3.047619       3.00      0.809776      0.507937
        Estr       6      3.047619       3.00      0.809776      0.507937


                      Average scores were used for ties.



                          Median One-Way Analysis

                      Chi-Square           9.4273
                      DF                        6
                      Pr > Chi-Square      0.1509
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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```
              Plot of METAMORP*CONC.  Symbol used is '*'.

METAMORP 52 ê
            é
            é   *
            é
            é
            é
            é
            é
         50 ê
            é
            é
            é
            é
            é
            é
            é        *
         48 ê
            é
            é
            é
            é
            é
            é
         46 ê
            é
            é
            é
            é
            é
            é                                                          *
         44 ê
            é
            é
            é
            é
            é
            é                          *
         42 ê
            é
            êâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâêâãâ
             0  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

                                    CONC
```
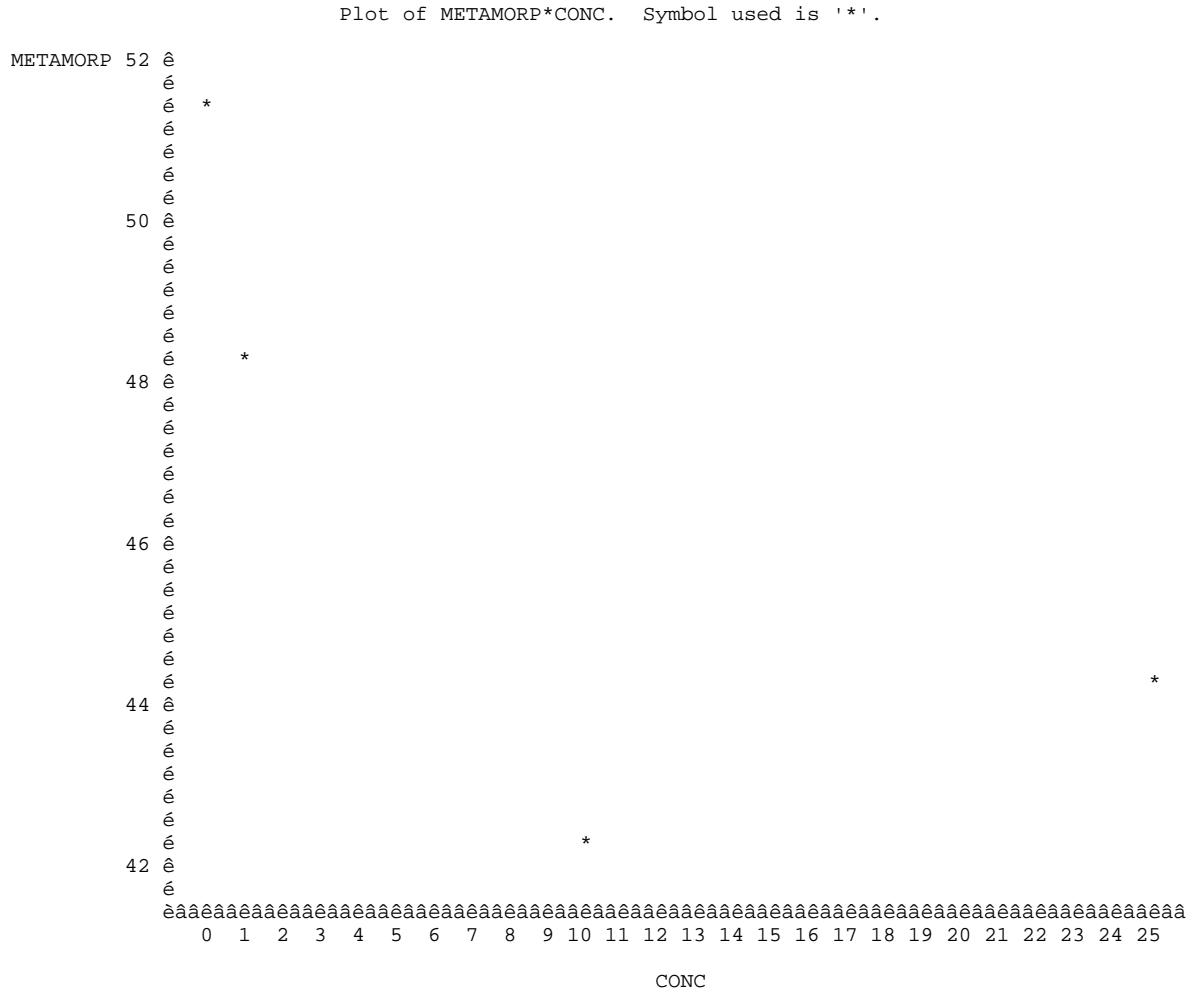
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

```
                        The CORR Procedure

                 1 With Variables:    CONC
                 1      Variables:    PMET


                        Simple Statistics

Variable        N       Mean       Std Dev         Sum      Minimum      Maximum

CONC           44    9.00000      10.14087   396.00000            0     25.00000
PMET           44   46.63390       9.96659        2052     19.67213     66.66667


              Pearson Correlation Coefficients, N = 44
                    Prob > |r| under H0: Rho=0

                                    PMET

              CONC       -0.24787
                           0.1047
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
                      Plot of SWIM*CONC.   Symbol used is '*'.

SWIM 4.0  é
          é
          é                                                                    *
          é
          é
          é
   3.5    ê
          é
          é
          é
          é
          é
          é                          *
          é
   3.0    ê
          é
          é
          é
          é
          é
          é
   2.5    ê
          é
          é
          é
          é
          é         *
          é
   2.0    ê
          é
          é
          é
          é
          é
          é
   1.5    ê
          é
          é   *
          é
          é
          é
   1.0    é
          êâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäêâäâ
           0  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

                                     CONC
    CORRELATION ANALYSIS OF PERCENT OF TADPOLES WITH ABNORMAL SWIMMING OVER CONCENTRATION   255

                              The CORR Procedure

                     1 With Variables:    CONC
                     1      Variables:    SWIM


                              Simple Statistics

  Variable          N       Mean      Std Dev         Sum    Minimum      Maximum

  CONC             44    9.00000     10.14087   396.00000          0     25.00000
  SWIM             44    2.59797      2.07806   114.31082          0      7.35294


                    Pearson Correlation Coefficients, N = 44
                         Prob > |r| under H0: Rho=0

                                    SWIM

                         CONC       0.40804
                                    0.0060
```
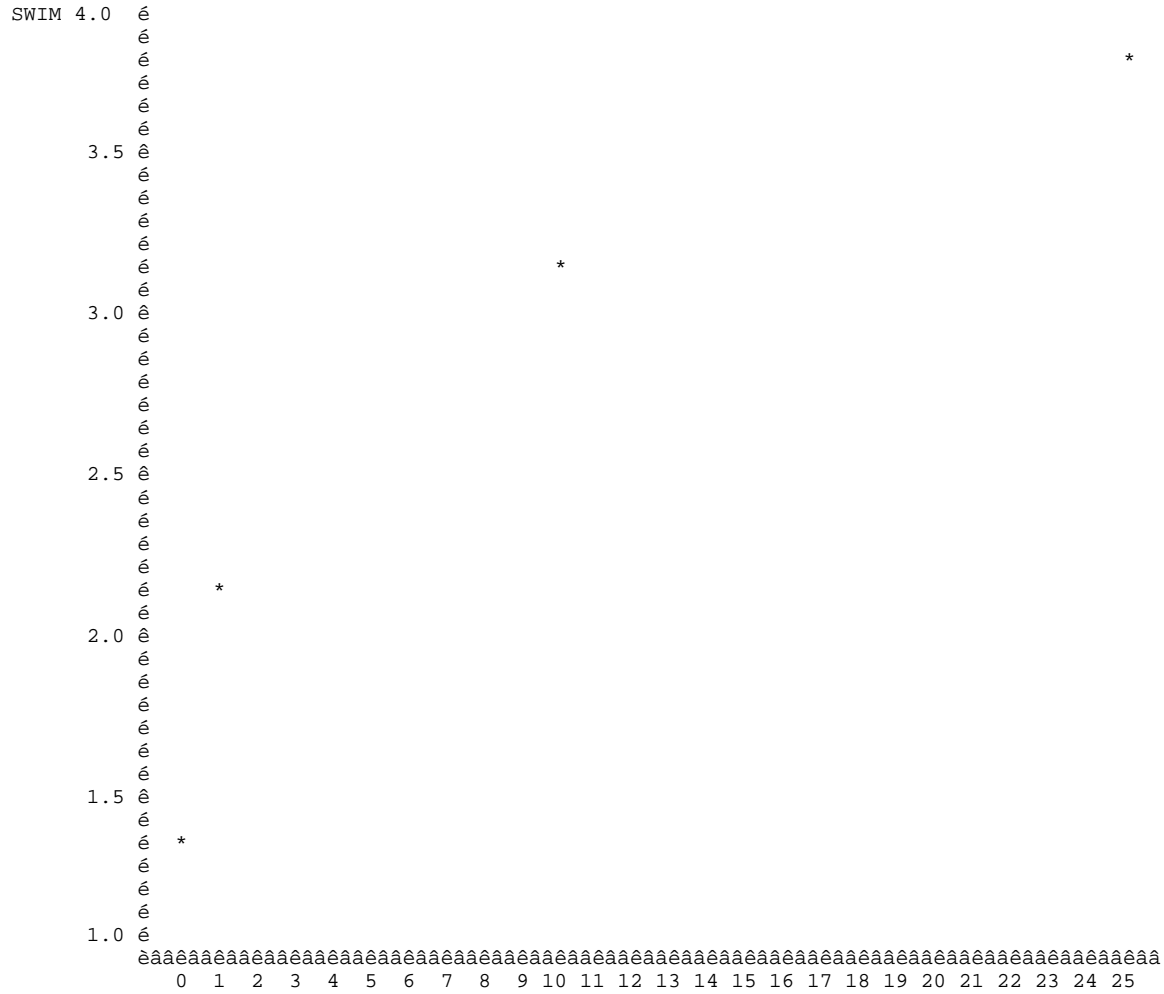
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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```
Plot of EDEMA*CONC.   Symbol used is '*'.
EDEMA 1.2 ê
          é
          é
          é
          é
          é
          é                                                              *
    1.0 ê
          é
          é
          é
          é
          é
          é
          é
    0.8 ê
          é
          é
          é
          é
          é
          é
    0.6 ê
          é
          é
          é
          é
          é                     *
    0.4 ê
          é
          é
          é        *
          é
          é
          é
    0.2 ê
          é
          é  *
          é
          é
          é
    0.0 ê
          êâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãêâãââ
           0  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
                                         CONC
        CORRELATION ANALYSIS OF PERCENT OF TADPOLES WITH EDEMA OVER CONCENTRATION          257

                               The CORR Procedure

                      1 With Variables:    CONC
                      1       Variables:   EDEMA


                             Simple Statistics

   Variable        N        Mean      Std Dev          Sum      Minimum      Maximum

   CONC           44     9.00000     10.14087    396.00000            0     25.00000
   EDEMA          44     0.48126      0.82662     21.17552            0      3.17460


                  Pearson Correlation Coefficients, N = 44
                      Prob > |r|  under H0: Rho=0

                                     EDEMA

                        CONC         0.40388
                                     0.0066
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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```
           Mean Body Weight of Frogs by Treatment                          1202

     Obs     treat    _TYPE_    _FREQ_      MEAN        STDERR

      1      0.0        0        335       0.32519    .005212036
      2      1.0        0        312       0.31670    .005086144
      3      10         0        277       0.34574    .006660663
      4      25         0        299       0.32173    .005397997
      5      DHT        0        135       0.32539    .008838205
      6      ETOH       0        161       0.31324    .007248717
      7      Estr       0        151       0.32720    .007594965
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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```
                    Mean Body Weight of Frogs by Treatment                         1203

                     Plot of MEAN*treat.   Symbol used is '*'.

    MEAN é
         é
   0.350 ê
         é
         é
         é
         é
         é                                  *
   0.345 ê
         é
         é
         é
         é
         é
   0.340 ê
         é
         é
         é
         é
   0.335 ê
         é
         é
         é
         é
   0.330 ê
         é
         é
         é                                                                    *
         é
   0.325 ê    *                                              *
         é
         é
         é                                        *
         é
   0.320 ê
         é
         é
         é              *
         é
   0.315 ê
         é
         é                                                         *
         é
         é
   0.310 ê
         é
         êâ âêâ â â â â â â â â â â â êâ â â â â â â â â â â â êâ â â â â â â â â â â â êâ â â â â â â â â â â â êâ â â â â â â â â â â â êâ â â â â â â â â â â â êâ â
            0.0           1.0           10            25           DHT          ETOH          Estr
                                                    treat
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

```
Analysis of Variance for Frog Body Weight over Treatment          1204

                        The GLM Procedure

                     Class Level Information

          Class      Levels   Values

          treat           7   0.0 1.0 10 25 DHT ETOH Estr


                 Number of observations    1670

NOTE: Due to missing values, only 1661 observations can be used in this analysis.

Dependent Variable: BW
```

Dependent Variable: BW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.16575046	0.02762508	2.96	0.0071
Error	1654	15.45132260	0.00934179		
Corrected Total	1660	15.61707306			

R-Square	Coeff Var	Root MSE	BW Mean
0.010613	29.69766	0.096653	0.325456

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	6	0.16575046	0.02762508	2.96	0.0071

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	6	0.16575046	0.02762508	2.96	0.0071

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
                Analysis of Variance for Frog Body Weight over Treatment              1206

                              The GLM Procedure

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

                             Sum of         Mean
        Source        DF     Squares       Square    F Value    Pr > F

        treat          6     0.00343     0.000571       1.40    0.2098
        Error       1654     0.6737      0.000407


                   Bartlett's Test for Homogeneity of BW Variance

                   Source        DF    Chi-Square    Pr > ChiSq

                   treat          6      17.8554        0.0066
                Analysis of Variance for Frog Body Weight over Treatment              1207

                              The GLM Procedure

                            Dunnett's t Tests for BW

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


                    Alpha                              0.05
                    Error Degrees of Freedom           1654
                    Error Mean Square               0.009342
                    Critical Value of Dunnett's t   2.59779


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

                              Difference
            treat             Between       Simultaneous 95%
          Comparison           Means        Confidence Limits

          10   - 0.0          0.020549      0.000124  0.040974   ***
          Estr - 0.0          0.002005     -0.022617  0.026628
          DHT  - 0.0          0.000197     -0.025410  0.025804
          25   - 0.0         -0.003465     -0.023509  0.016578
          1.0  - 0.0         -0.008491     -0.028309  0.011328
          ETOH - 0.0         -0.011958     -0.036098  0.012183
```
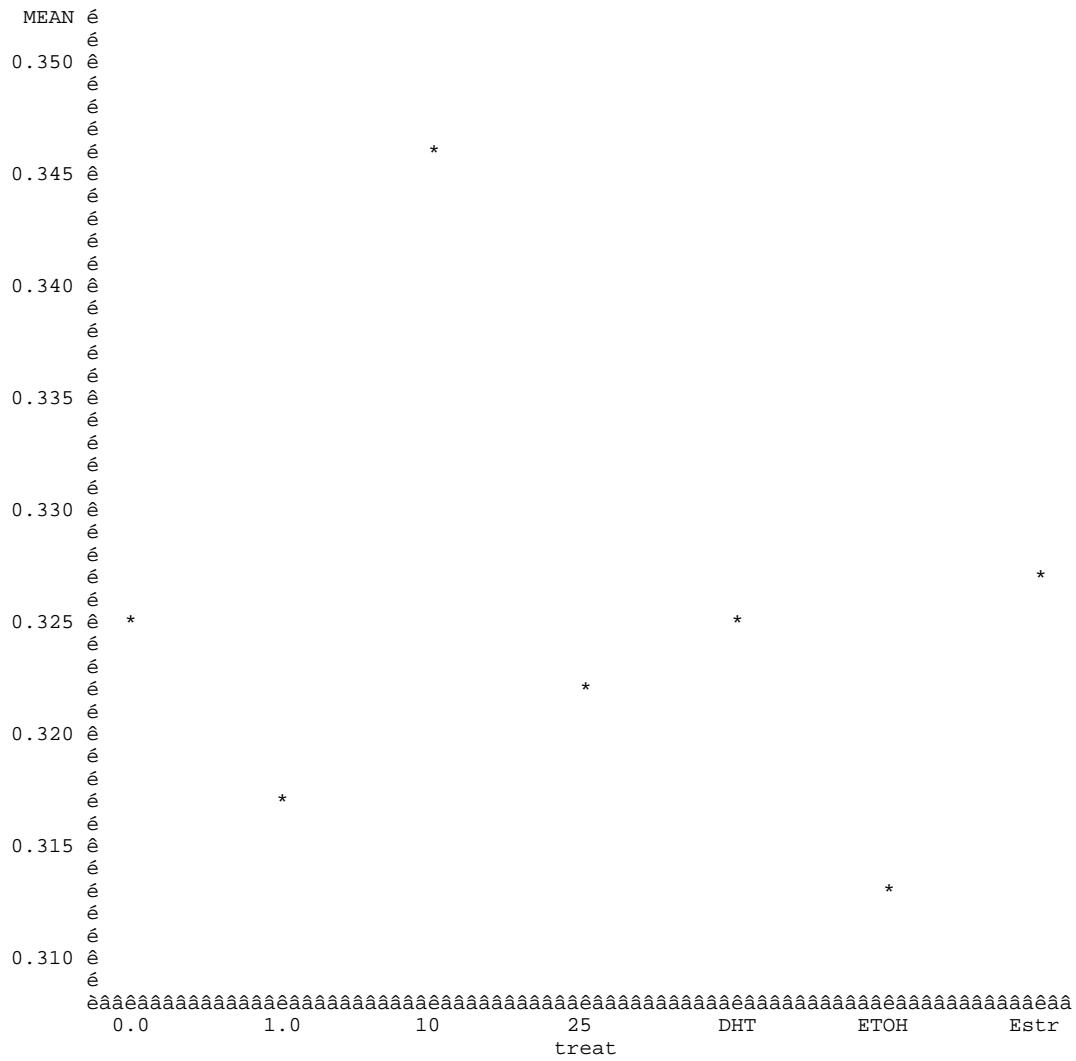
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
            Nonparametric comparison of frog body weight over treatments          1208

                               The NPAR1WAY Procedure

                      Wilcoxon Scores (Rank Sums) for Variable BW
                             Classified by Variable treat

                           Sum of      Expected       Std Dev          Mean
        treat      N       Scores      Under H0       Under H0         Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0      334    279296.50     277554.0     7834.89949     836.217066
        1.0      309    244939.50     256779.0     7606.63049     792.684466
        10       276    254956.50     229356.0     7276.19220     923.755435
        25       296    242002.00     245976.0     7480.60832     817.574324
        DHT      135    109378.50     112185.0     5341.56953     810.211111
        ETOH     160    122301.50     132960.0     5767.33359     764.384375
        Estr     151    127416.50     125481.0     5619.55184     843.817881
                        Average scores were used for ties.


                               Kruskal-Wallis Test

                        Chi-Square          16.0133
                        DF                        6
                        Pr > Chi-Square      0.0137


           Median Scores (Number of Points Above Median) for Variable BW
                             Classified by Variable treat

                           Sum of      Expected       Std Dev          Mean
        treat      N       Scores      Under H0       Under H0         Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0      334      178.0     166.899458     8.170047      0.532934
        1.0      309      143.0     154.406984     7.932014      0.462783
        10       276      162.0     137.916918     7.587441      0.586957
        25       296      141.0     147.910897     7.800601      0.476351
        DHT      135       61.0      67.459362     5.570062      0.451852
        ETOH     160       67.0      79.951836     6.014039      0.418750
        Estr     151       78.0      75.454545     5.859935      0.516556


                        Average scores were used for ties.


                               Median One-Way Analysis

                        Chi-Square          17.8022
                        DF                        6
                        Pr > Chi-Square      0.0067
            Average Snout to Vent Length of Frogs by Treatment          1210

            Obs     treat    _TYPE_    _FREQ_      MEAN      STDERR

             1      0.0        0         335     13.9566    0.08149
             2      1.0        0         312     13.7896    0.07939
             3      10         0         277     14.0815    0.09106
             4      25         0         299     13.9865    0.09052
             5      DHT        0         135     13.9963    0.13501
             6      ETOH       0         161     13.5938    0.10530
             7      Estr       0         151     14.2914    0.11416
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
          Average Snout to Vent Length of Frogs by Treatment                    1211

              Plot of MEAN*treat.  Symbol used is '*'.

MEAN é
14.3 ê
     é                                                              *
     é
     é
     é
     é
     é
14.2 ê
     é
     é
     é
     é
     é
14.1 ê
     é                             *
     é
     é
     é
     é
14.0 ê
     é                                          *
     é                                 *
     é   *
     é
     é
13.9 ê
     é
     é
     é
     é
     é
13.8 ê
     é              *
     é
     é
     é
     é
13.7 ê
     é
     é
     é
     é
     é
13.6 ê                                                  *
     é
     êâãêâãâãâãâãâãâãâãâãêâãâãâãâãâãâãâãêâãâãâãâãâãâãâãêâãâãâãâãâãâãâãêâãâãâãâãâãâãâãêâãâãâãâãâãâãêâãâã
         0.0         1.0         10          25         DHT        ETOH        Estr

                                  treat
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
        Analysis of Variance for Frog Snout to Vent Length over Treatment        1212

                            The GLM Procedure

                          Class Level Information
            Class         Levels    Values
            treat           7      0.0 1.0 10 25 DHT ETOH Estr


                      Number of observations    1670

NOTE: Due to missing values, only 1661 observations can be used in this analysis.

Dependent Variable: SVL

                                    Sum of
      Source                 DF      Squares     Mean Square    F Value    Pr > F
      Model                   6     51.322535       8.553756       3.94    0.0006
      Error                1654   3590.329783       2.170695
      Corrected Total      1660   3641.652318


            R-Square      Coeff Var      Root MSE      SVL Mean

            0.014093      10.56124       1.473328      13.95033


      Source                 DF     Type I SS      Mean Square    F Value    Pr > F
      treat                   6   51.32253515       8.55375586       3.94    0.0006

      Source                 DF    Type III SS     Mean Square    F Value    Pr > F
      treat                   6   51.32253515       8.55375586       3.94    0.0006

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

                            Sum of        Mean
            Source     DF    Squares      Square     F Value    Pr > F

            treat       6    81.5820     13.5970       1.10     0.3612
            Error    1654    20484.3     12.3847


            Bartlett's Test for Homogeneity of SVL Variance

            Source     DF    Chi-Square    Pr > ChiSq

            treat       6      8.9126        0.1786

                       Dunnett's t Tests for SVL

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


                    Alpha                          0.05
                    Error Degrees of Freedom       1654
                    Error Mean Square          2.170695
                    Critical Value of Dunnett's t  2.59779


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


                        Difference     Simultaneous
              treat        Between     95% Confidence
            Comparison      Means          Limits

            Estr - 0.0      0.3348    -0.0405   0.7101
            10   - 0.0      0.1249    -0.1864   0.4363
            DHT  - 0.0      0.0397    -0.3506   0.4301
            25   - 0.0      0.0299    -0.2756   0.3354
            1.0  - 0.0     -0.1669    -0.4690   0.1352
            ETOH - 0.0     -0.3628    -0.7308   0.0052
```
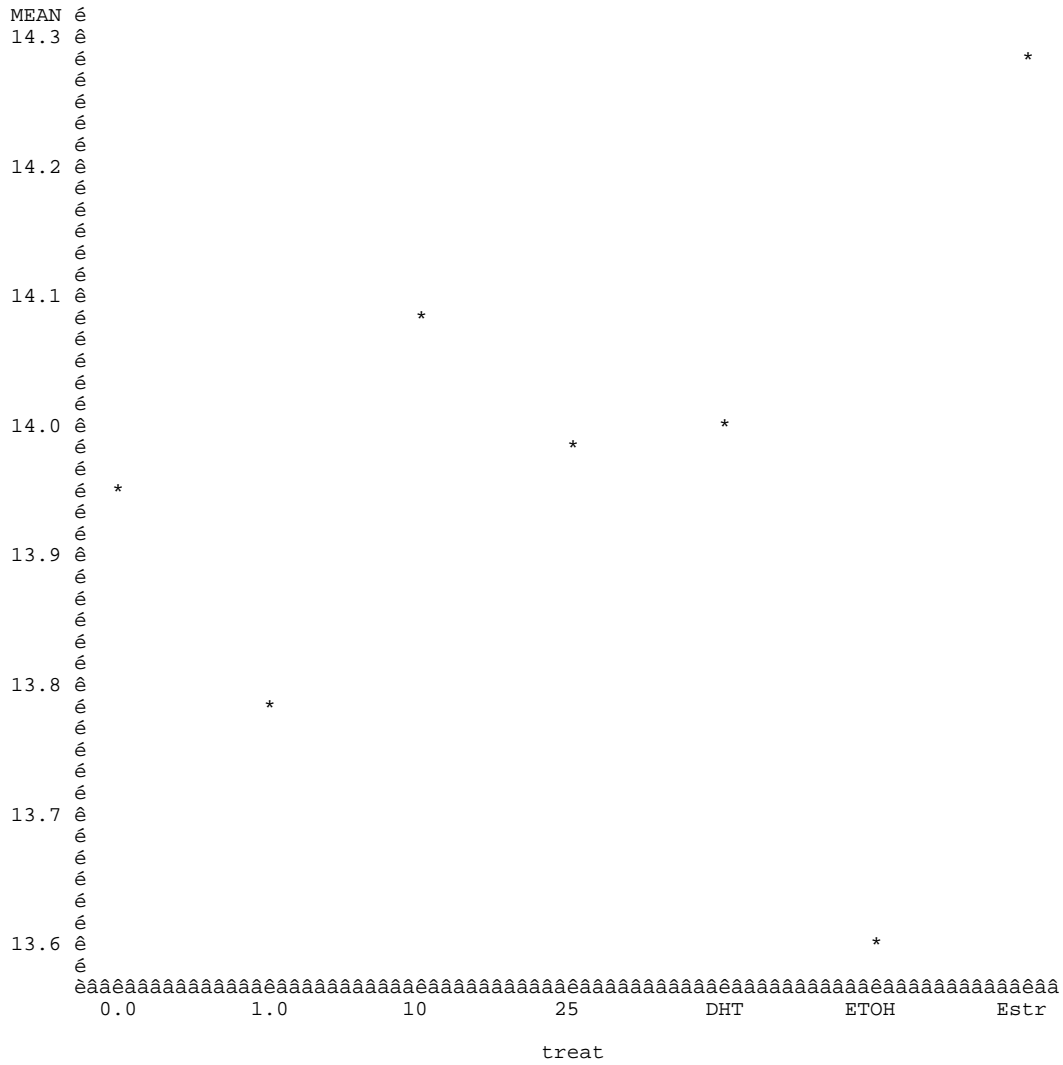
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
              Nonparametric comparison of frog length over treatments              1216

                              The NPAR1WAY Procedure

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

                         Sum of      Expected       Std Dev          Mean
        treat      N     Scores      Under H0       Under H0         Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0      334    277451.50    277554.0      7761.12060     830.693114
        1.0      309    243946.50    256779.00115   7535.00115     789.470874
        10       276    241172.50    229356.0      7207.67449     873.813406
        25       296    247542.00    245976.0      7410.16568     836.290541
        DHT      135    113146.50    112185.0      5291.26958     838.122222
        ETOH     160    114795.50    132960.0      5713.02434     717.471875
        Estr     151    142236.50    125481.0      5566.63421     941.963576
                      Average scores were used for ties.

                              Kruskal-Wallis Test

                        Chi-Square          22.0409
                        DF                        6
                        Pr > Chi-Square      0.0012

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

                         Sum of      Expected       Std Dev          Mean
        treat      N     Scores      Under H0       Under H0         Score
        âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
        0.0      334    166.322485   166.899458    7.423923       0.497972
        1.0      309    141.224852   154.406984    7.207627       0.457038
        10       276    150.535503   137.916918    6.894522       0.545418
        25       296    150.807692   147.910897    7.088216       0.509485
        DHT      135     68.059172    67.459362    5.061379       0.504142
        ETOH     160     65.059172    79.951836    5.464810       0.406620
        Estr     151     87.991124    75.454545    5.324780       0.582723

                      Average scores were used for ties.


                            Median One-Way Analysis

                        Chi-Square          17.4213
                        DF                        6
                        Pr > Chi-Square      0.0079
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
                      Percentage of Frogs Male or Female                            1218

---------------------------------------- treat=0.0 ----------------------------------------

                              The FREQ Procedure

                                          Cumulative    Cumulative
         Sex     Frequency      Percent    Frequency      Percent
         âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
         ?            2          0.60            2          0.60
         F          168         50.30          170         50.90
         M          164         49.10          334        100.00

                          Frequency Missing = 1
                      Percentage of Frogs Male or Female                            1219

---------------------------------------- treat=1.0 ----------------------------------------

                              The FREQ Procedure

                                          Cumulative    Cumulative
         Sex     Frequency      Percent    Frequency      Percent
         âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
         ?            3          0.97            3          0.97
         F          152         49.19          155         50.16
         M          154         49.84          309        100.00

                          Frequency Missing = 3
                      Percentage of Frogs Male or Female                            1220

----------------------------------------- treat=10 -----------------------------------------

                              The FREQ Procedure

                                          Cumulative    Cumulative
         Sex     Frequency      Percent    Frequency      Percent
         âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
         ?            1          0.36            1          0.36
         F          142         51.45          143         51.81
         M          133         48.19          276        100.00

                          Frequency Missing = 1
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

Percentage of Frogs Male or Female   1221

-- treat=25 --

The FREQ Procedure

| | | | Cumulative | Cumulative |
Sex	Frequency	Percent	Frequency	Percent
?	14	4.73	14	4.73
F	149	50.34	163	55.07
M	133	44.93	296	100.00

Frequency Missing = 3

Percentage of Frogs Male or Female   1222

-- treat=DHT --

The FREQ Procedure

| | | | Cumulative | Cumulative |
Sex	Frequency	Percent	Frequency	Percent
F	73	54.07	73	54.07
M	62	45.93	135	100.00

Percentage of Frogs Male or Female   1223

-- treat=ETOH --

The FREQ Procedure

| | | | Cumulative | Cumulative |
Sex	Frequency	Percent	Frequency	Percent
F	81	50.63	81	50.63
M	79	49.38	160	100.00

Frequency Missing = 1

Percentage of Frogs Male or Female   1224

-- treat=Estr --

The FREQ Procedure

| | | | Cumulative | Cumulative |
Sex	Frequency	Percent	Frequency	Percent
?	11	7.28	11	7.28
F	101	66.89	112	74.17
M	39	25.83	151	100.00

Average Total Area of Frog Larynx by Treatment   1225

Obs	treat	_TYPE_	_FREQ_	MEAN	STDERR
1	0.0	0	56	0.14694	0.003893
2	1.0	0	51	0.15720	0.005215
3	10	0	55	0.15998	0.003926
4	25	0	54	0.15752	0.004347
5	DHT	0	12	0.30669	0.012958
6	ETOH	0	12	0.14100	0.009301
7	Estr	0	12	0.14480	0.006760

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
                           Plot of MEAN*treat.   Symbol used is '*'.

      MEAN é
           é
    0.325  ê
           é
           é
           é
           é                                                          *
    0.300  ê
           é
           é
           é
           é
    0.275  ê
           é
           é
           é
           é
    0.250  ê
           é
           é
           é
           é
    0.225  ê
           é
           é
           é
           é
    0.200  ê
           é
           é
           é
           é
    0.175  ê
           é
           é
           é                            *              *
           é                    *
    0.150  ê
           é    *
           é                                                                      *
           é                                                      *
           é
    0.125  ê
           é
           êâãêâââââââââââââêâââââââââââââêâââââââââââââêâââââââââââââêâââââââââââââêââââââââââââêââ
             0.0           1.0           10            25           DHT          ETOH          Estr

                                                 treat
```
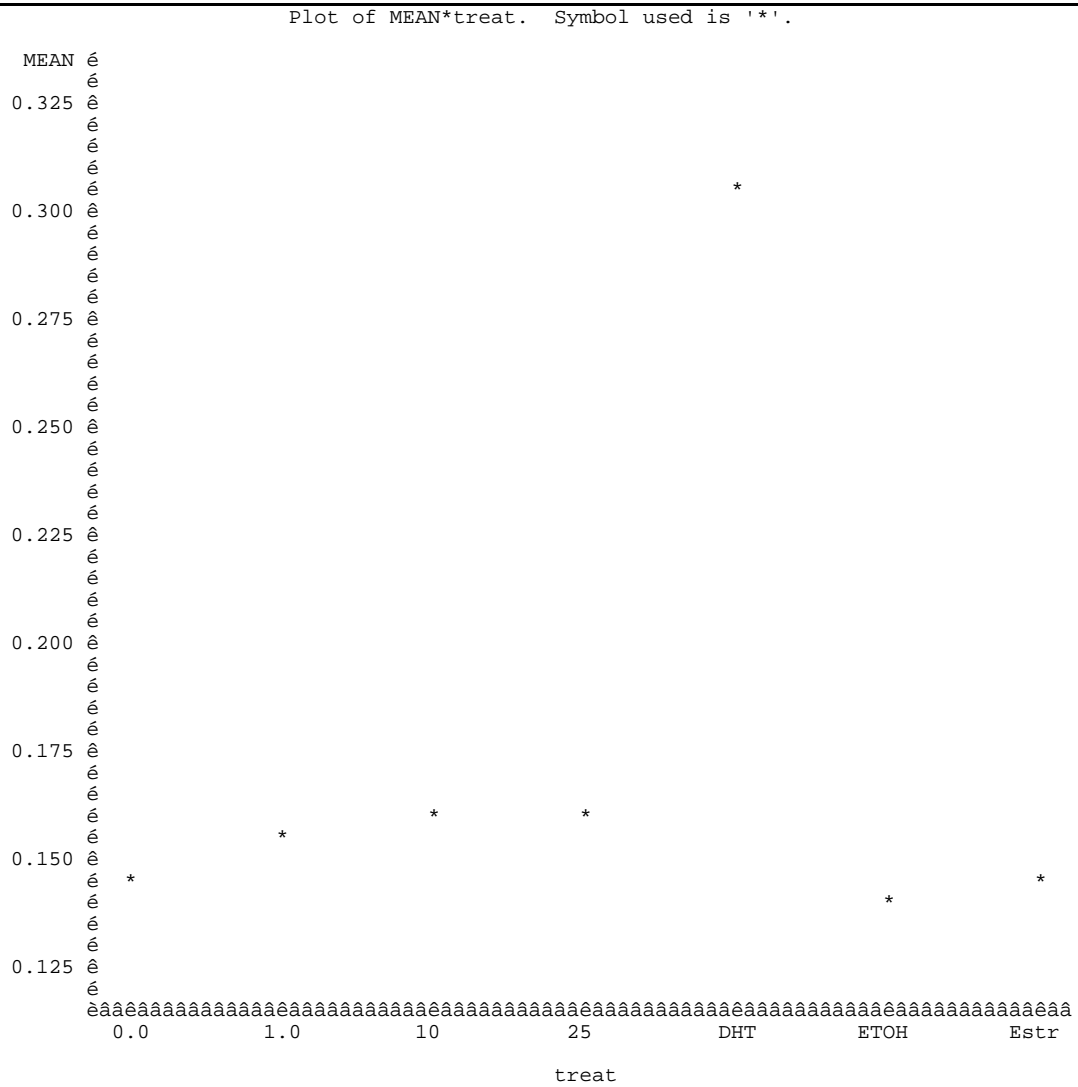
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
           Analysis of Variance for Larynx Total Area over Treatment            1227

                              The GLM Procedure

                           Class Level Information

                    Class       Levels    Values
                    treat          7       0.0 1.0 10 25 DHT ETOH Estr


                      Number of observations    252

Dependent Variable: TOTAL

                                      Sum of
        Source                  DF     Squares      Mean Square    F Value    Pr > F
        Model                    6    0.27515908     0.04585985      43.98    <.0001
        Error                  245    0.25549096     0.00104282
        Corrected Total        251    0.53065004


               R-Square      Coeff Var       Root MSE     TOTAL Mean

               0.518532      20.01396        0.032293       0.161351


        Source                  DF      Type I SS     Mean Square    F Value    Pr > F
        treat                    6     0.27515908     0.04585985      43.98    <.0001

        Source                  DF     Type III SS    Mean Square    F Value    Pr > F
        treat                    6     0.27515908     0.04585985      43.98    <.0001

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

                               Sum of         Mean
                Source     DF   Squares       Square    F Value    Pr > F

                treat       6   0.000021      3.543E-6     1.82     0.0955
                Error     245   0.000477      1.945E-6


                 Bartlett's Test for Homogeneity of TOTAL Variance

                   Source       DF    Chi-Square    Pr > ChiSq

                   treat         6      9.1161         0.1672

                      Dunnett's t Tests for TOTAL

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


                    Alpha                            0.05
                    Error Degrees of Freedom          245
                    Error Mean Square            0.001043
                    Critical Value of Dunnett's t  2.62465


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


                          Difference
              treat        Between      Simultaneous 95%
           Comparison       Means      Confidence Limits

           DHT  - 0.0      0.159753      0.132791  0.186715   ***
           10   - 0.0      0.013036     -0.003054  0.029126
           25   - 0.0      0.010574     -0.005591  0.026739
           1.0  - 0.0      0.010257     -0.006148  0.026663
           Estr - 0.0     -0.002144     -0.029106  0.024817
           ETOH - 0.0     -0.005940     -0.032901  0.021022
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
                      The NPAR1WAY Procedure

            Wilcoxon Scores (Rank Sums) for Variable TOTAL
                     Classified by Variable treat

                     Sum of      Expected      Std Dev        Mean
treat      N         Scores      Under H0      Under H0       Score
âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
0.0        56         5953.0      7084.00      481.051626   106.303571
1.0        51         6269.0      6451.50      464.892730   122.921569
10         55         7427.0      6957.50      477.951793   135.036364
25         54         6899.0      6831.00      474.787321   127.759259
DHT        12         2956.0      1518.00      246.414285   246.333333
ETOH       12         1161.0      1518.00      246.414285    96.750000
Estr       12         1213.0      1518.00      246.414285   101.083333


                       Kruskal-Wallis Test

                  Chi-Square          41.0845
                  DF                        6
                  Pr > Chi-Square      <.0001

   Median Scores (Number of Points Above Median) for Variable TOTAL
                     Classified by Variable treat

                     Sum of      Expected      Std Dev        Mean
treat      N         Scores      Under H0      Under H0       Score
âââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââââ
0.0        56          23.0        28.00       3.306398     0.410714
1.0        51          24.0        25.50       3.195334     0.470588
10         55          32.0        27.50       3.285092     0.581818
25         54          25.0        27.00       3.263342     0.462963
DHT        12          12.0         6.00       1.693672     1.000000
ETOH       12           5.0         6.00       1.693672     0.416667
Estr       12           5.0         6.00       1.693672     0.416667


                       Median One-Way Analysis

                  Chi-Square          16.3328
                  DF                        6
                  Pr > Chi-Square      0.0121
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
          Analysis of Variance for Larynx Total Area over Treatment by Sex        1233

-------------------------------------- Sex=F --------------------------------------

                                The GLM Procedure

                             Class Level Information

                    Class       Levels   Values

                    treat          7    0.0 1.0 10 25 DHT ETOH Estr


                          Number of observations    62
Dependent Variable: TOTAL

                                       Sum of
     Source                    DF       Squares      Mean Square   F Value   Pr > F
     Model                      6    0.19693080       0.03282180     43.04   <.0001
     Error                     55    0.04194645       0.00076266
     Corrected Total           61    0.23887726


            R-Square     Coeff Var      Root MSE     TOTAL Mean

            0.824402     19.46572       0.027616      0.141872


     Source                    DF       Type I SS     Mean Square   F Value   Pr > F
     treat                      6    0.19693080       0.03282180     43.04   <.0001

     Source                    DF      Type III SS    Mean Square   F Value   Pr > F
     treat                      6    0.19693080       0.03282180     43.04   <.0001

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

                              Sum of        Mean
            Source       DF   Squares      Square    F Value   Pr > F

            treat         6   0.000034    5.612E-6      5.62   0.0001
            Error        55   0.000055    9.985E-7


              Bartlett's Test for Homogeneity of TOTAL Variance

                  Source       DF    Chi-Square    Pr > ChiSq

                  treat         6     16.3229        0.0121

                       Dunnett's t Tests for TOTAL

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


                   Alpha                              0.05
                   Error Degrees of Freedom             55
                   Error Mean Square              0.000763
                   Critical Value of Dunnett's t  2.67093


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


                         Difference
                 treat     Between    Simultaneous 95%
               Comparison    Means   Confidence Limits

               DHT  - 0.0   0.19547    0.15804  0.23291   ***
               Estr - 0.0   0.02421   -0.01322  0.06165
               10   - 0.0   0.01164   -0.01981  0.04310
               25   - 0.0   0.00455   -0.02624  0.03534
               ETOH - 0.0   0.00380   -0.03364  0.04123
               1.0  - 0.0   0.00047   -0.03176  0.03269
```

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Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

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Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

```
             Analysis of Variance for Larynx Total Area over Treatment by Sex          1237

------------------------------------- Sex=M -------------------------------------------

                                The GLM Procedure

                              Class Level Information

                   Class        Levels    Values

                   treat             7    0.0 1.0 10 25 DHT ETOH Estr


                          Number of observations    190

Dependent Variable: TOTAL

                                      Sum of
      Source                   DF     Squares     Mean Square    F Value   Pr > F
      Model                     6    0.11691360    0.01948560      24.82   <.0001
      Error                   183    0.14365715    0.00078501
      Corrected Total         189    0.26057076


            R-Square     Coeff Var      Root MSE     TOTAL Mean

            0.448683     16.70652      0.028018       0.167707


      Source                   DF    Type I SS     Mean Square    F Value   Pr > F
      treat                     6    0.11691360    0.01948560      24.82   <.0001

      Source                   DF    Type III SS   Mean Square    F Value   Pr > F
      treat                     6    0.11691360    0.01948560      24.82   <.0001
                 Levene's Test for Homogeneity of TOTAL Variance
               ANOVA of Squared Deviations from Group Means

                              Sum of        Mean
            Source      DF    Squares       Square     F Value   Pr > F

            treat        6    9.284E-6     1.547E-6       1.20   0.3067
            Error      183    0.000235     1.286E-6


              Bartlett's Test for Homogeneity of TOTAL Variance

              Source         DF    Chi-Square    Pr > ChiSq

              treat           6      6.4494        0.3748

                       Dunnett's t Tests for TOTAL

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


                   Alpha                            0.05
                   Error Degrees of Freedom          183
                   Error Mean Square            0.000785
                   Critical Value of Dunnett's t  2.63648


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


                          Difference
                  treat     Between      Simultaneous 95%
                Comparison    Means     Confidence Limits

                DHT  - 0.0    0.146489    0.114385  0.178594   ***
                10   - 0.0    0.013549   -0.002112  0.029210
                25   - 0.0    0.013522   -0.002327  0.029371
                1.0  - 0.0    0.012629   -0.003319  0.028578
                ETOH - 0.0    0.006778   -0.025327  0.038882
```

Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

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Estr - 0.0      -0.006049   -0.038154  0.026055
```