

US EPA ARCHIVE DOCUMENT

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

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

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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.

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.

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 archived.

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

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 Holtfretter’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 ± 1°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.	static renewal NA
Renewal rate for static renewal	50% exposure solution change every 72 hours

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

Parameter	Details
Aeration, if any	not reported
<u>Test vessel</u> 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
<u>Water parameters:</u> 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 22.8°C 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 μ g/mL) positive controls.

US EPA ARCHIVE DOCUMENT

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 ₂ O 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)

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

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, $p =$

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 dihydrotestosterone 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

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 \pm 0.004 (n = 45)	0.117 \pm 0.006 (n = 11)
1 μg atrazine/L	0.167 \pm 0.005 (n = 41)	0.118 \pm 0.006 (n = 10)
10 μg atrazine/L	0.168 \pm 0.004 (n = 44)	0.129 \pm 0.007 (n = 11)
25 μg atrazine/L	0.168 \pm 0.004 (n = 42)	0.122 \pm 0.006 (n = 12)
solvent control (0.0025% ethanol)	0.161 \pm 0.008 (n = 6)	0.121 \pm 0.013 (n = 6)
dihydrotestosterone	0.301 \pm 0.012* (n = 6)	0.313 \pm 0.024* (n = 6)
17- β estradiol	0.148 \pm 0.010 (n = 6)	0.141 \pm 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 greater 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 $\mu\text{g}/\text{L}$ 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.

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

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

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

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 μg /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.

H. REFERENCES:

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

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

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

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

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

EPA MRID Number 458677-07

NONPARAMETRIC COMPARISON OF PERCENT METAMORPHOSIS ACROSS TREATMENTS

242

The NPARIWAY Procedure

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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

US EPA ARCHIVE DOCUMENT

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 PERCENT SURVIVAL ACROSS TREATMENTS

244

The NPARIWAY 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

US EPA ARCHIVE DOCUMENT

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 PERCENT OF TADPOLES WITH ABNORMAL SWIMMING ACROSS TREATMENTS 246

The NPARIWAY Procedure

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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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 NPARIWAY Procedure

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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

EPA MRID Number 458677-07

Pr > Chi-Square 0.0975

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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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.

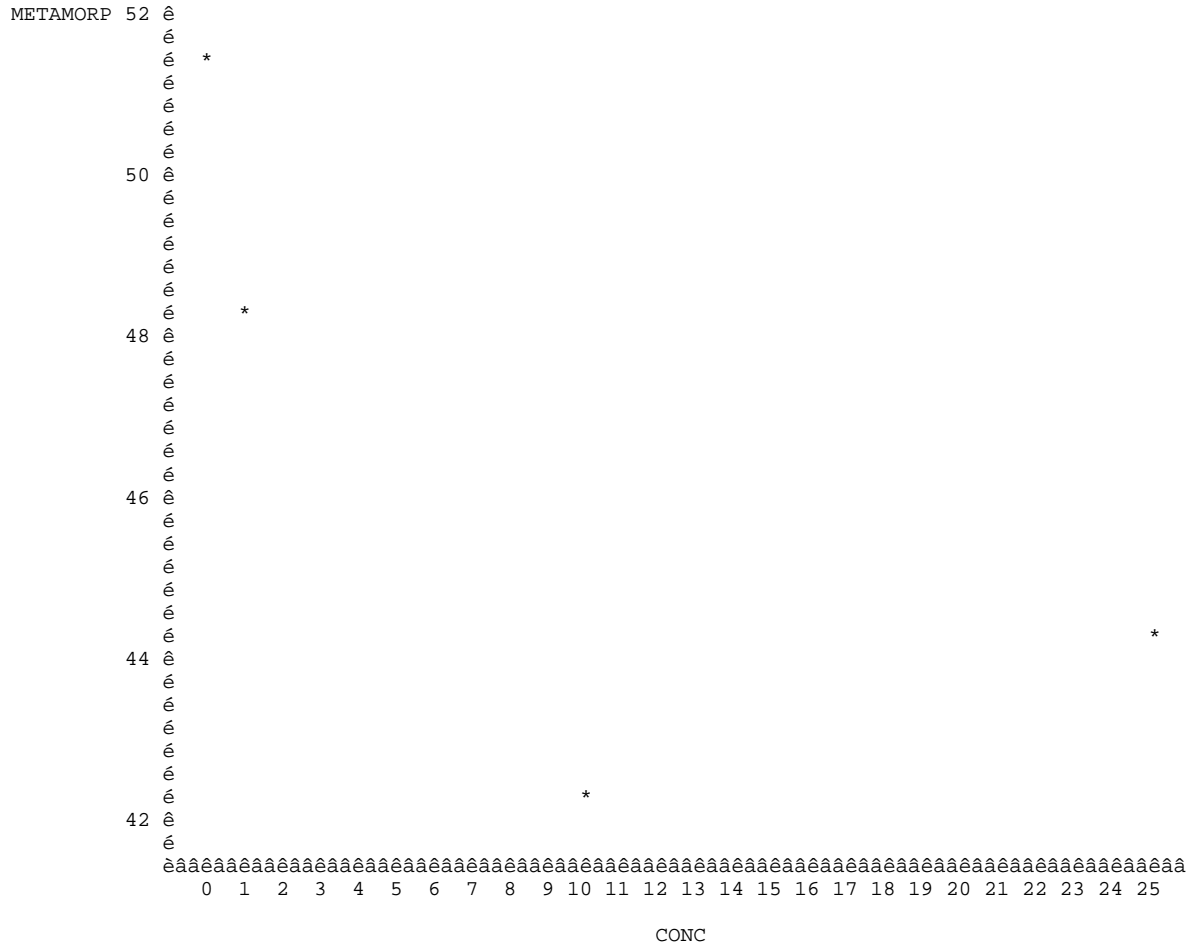
Median One-Way Analysis

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

EPA MRID Number 458677-07

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



US EPA ARCHIVE DOCUMENT

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

CORRELATION ANALYSIS OF PERCENT METAMORPHOSIS OVER CONCENTRATION

253

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

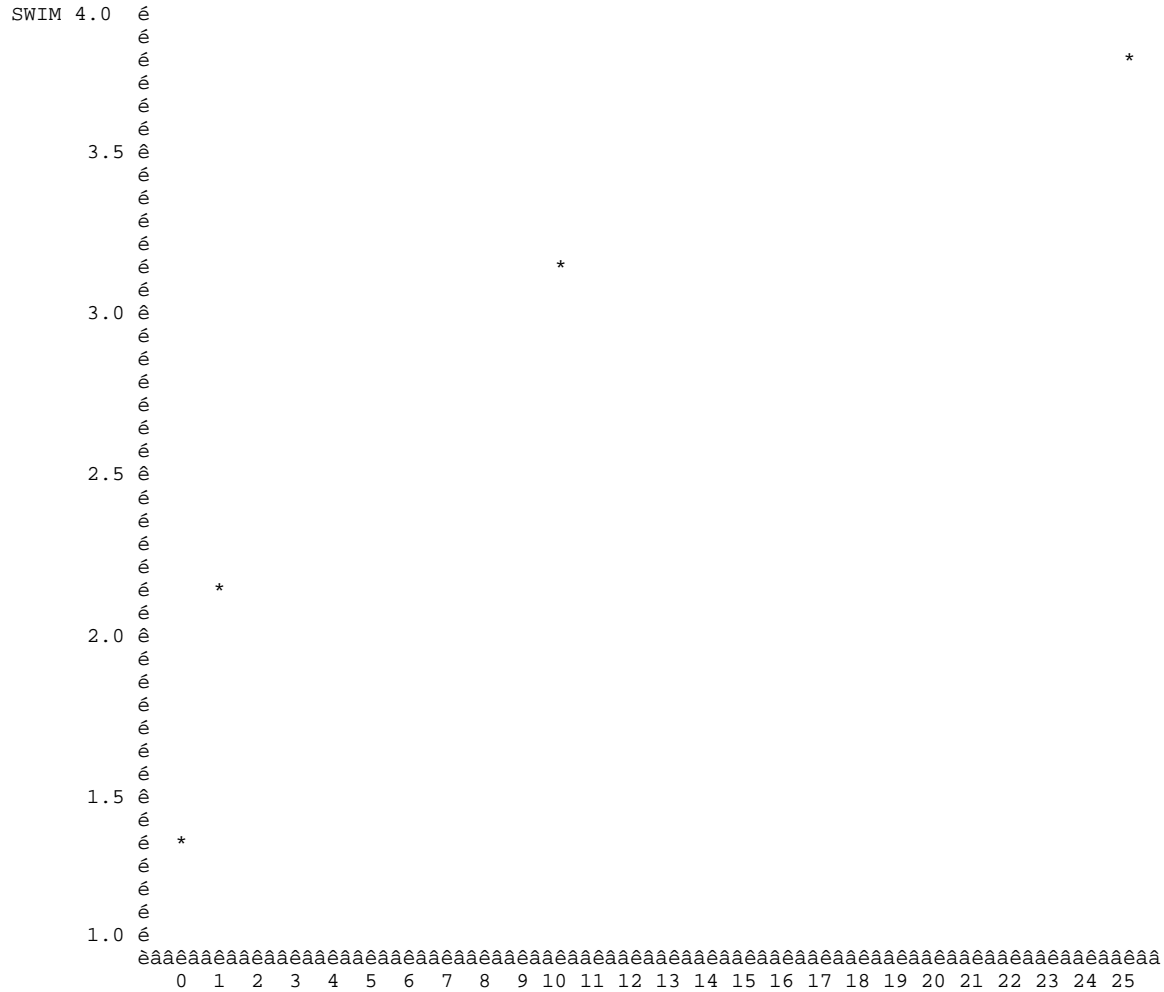
Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	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 '*'.



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

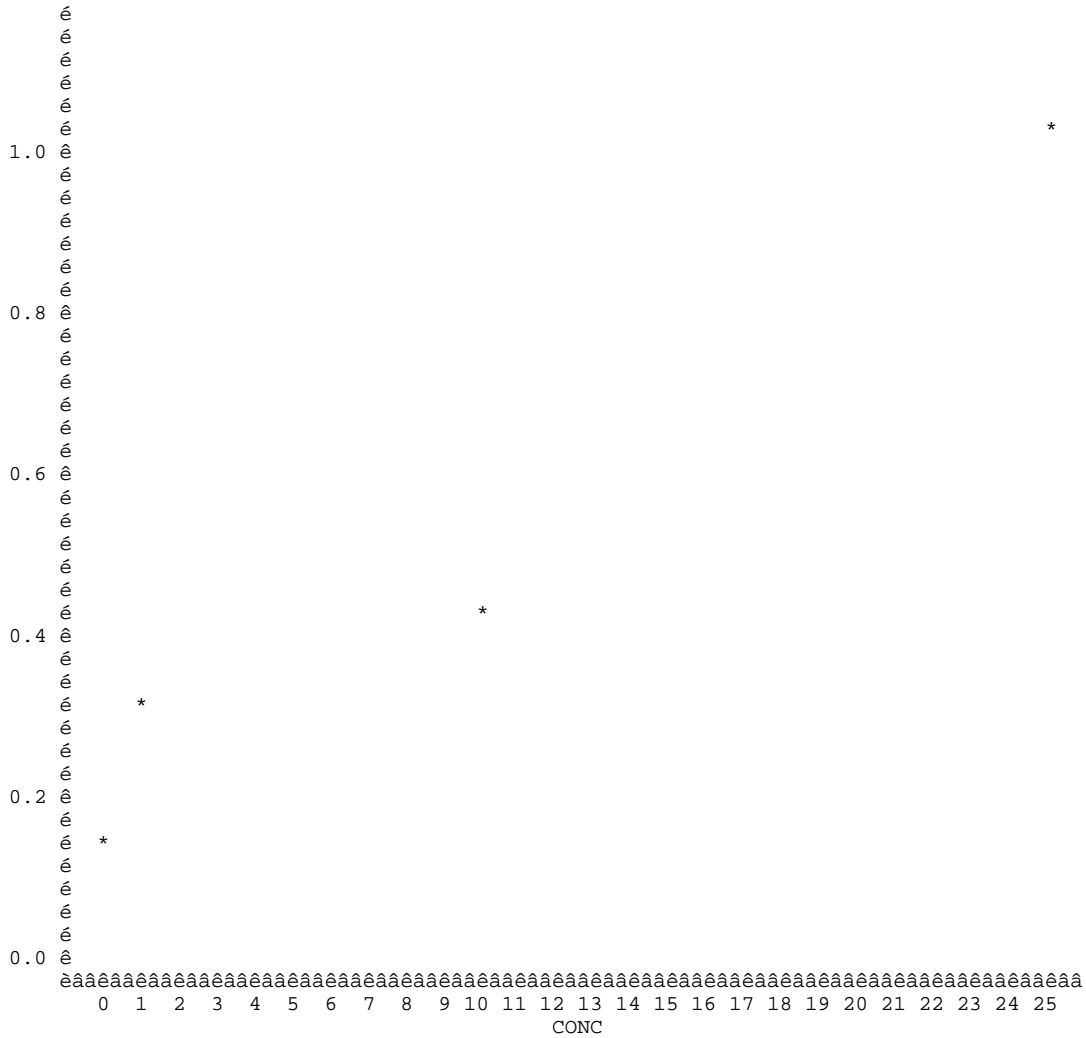
Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

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

EPA MRID Number 458677-07

Plot of EDEMA*CONC. Symbol used is '*'.
EDEMA 1.2 @



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

EPA MRID Number 458677-07

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

US EPA ARCHIVE DOCUMENT

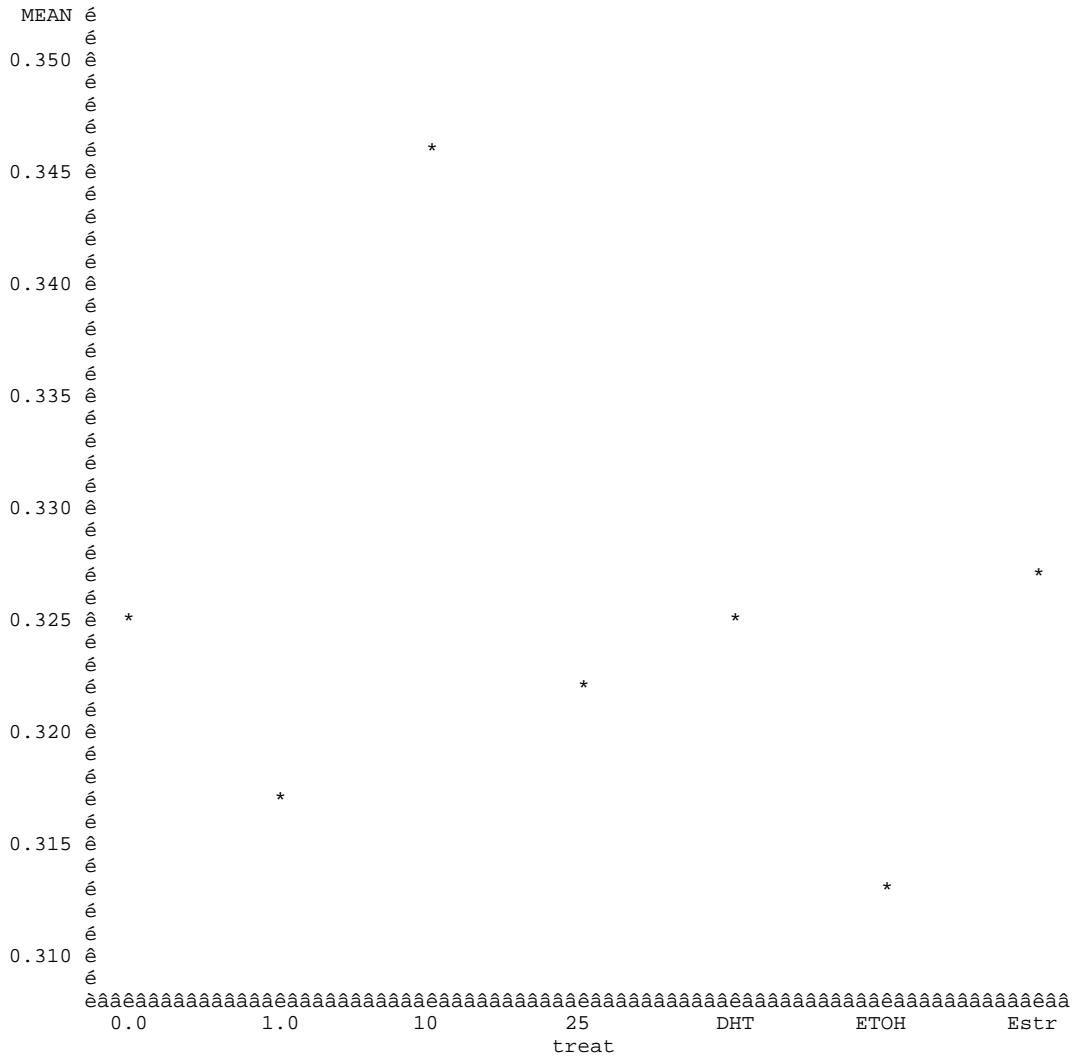
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

Mean Body Weight of Frogs by Treatment

1203

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



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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 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

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

Source	DF	Sum of Squares	Mean 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 ***.

treat Comparison	Difference Between Means	Simultaneous 95% 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 NPARIWAY Procedure

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

treat	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

treat	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

US EPA ARCHIVE DOCUMENT

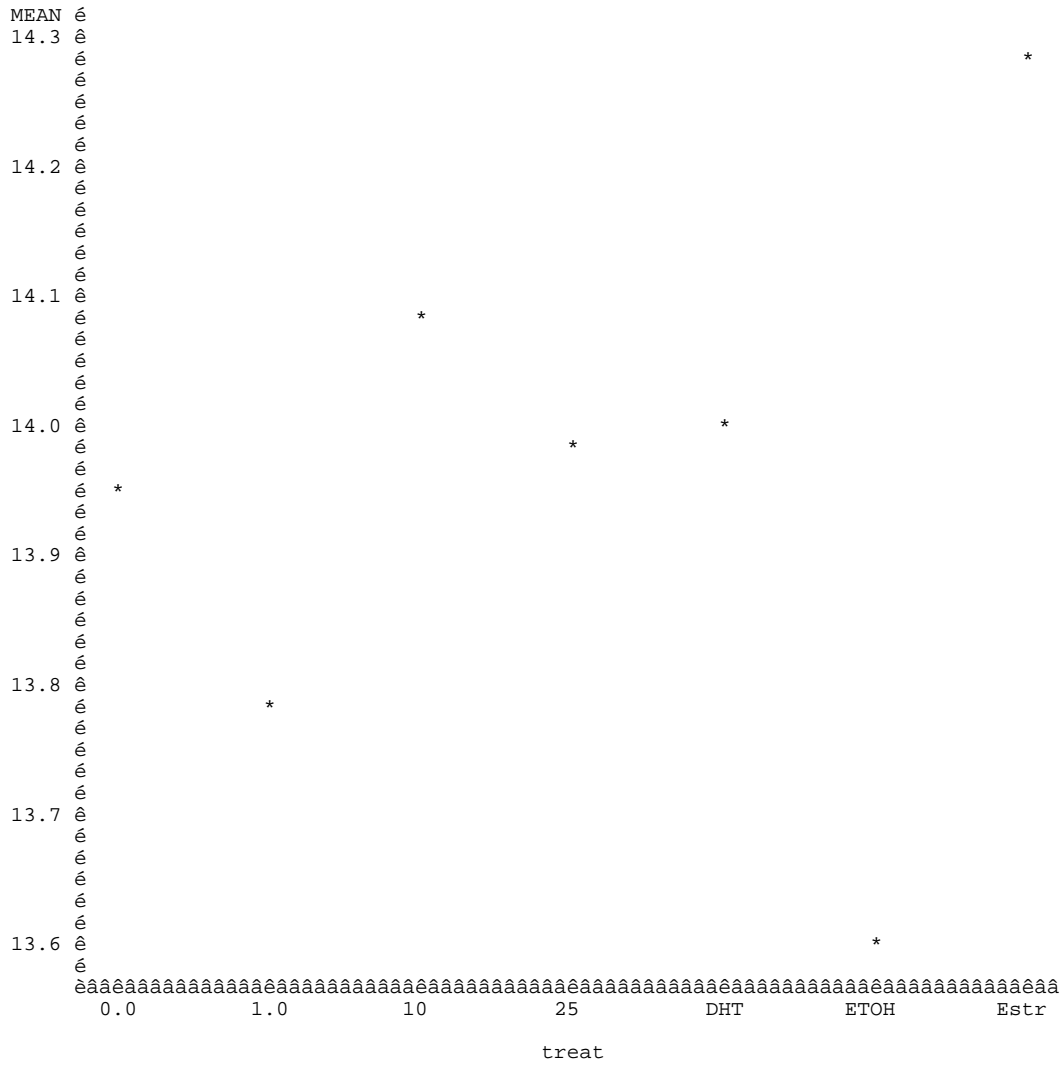
Data Evaluation Report on Response of Larval *Xenopus laevis* to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology

EPA MRID Number 458677-07

Average Snout to Vent Length of Frogs by Treatment

1211

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



US EPA ARCHIVE DOCUMENT

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

Source	DF	Sum of 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

Source	DF	Sum of Squares	Mean 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 ***.

treat Comparison	Difference Between Means	Simultaneous 95% Confidence 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

treat	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0.0	334	277451.50	277554.0	7761.12060	830.693114
1.0	309	243946.50	256779.0	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

treat	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

US EPA ARCHIVE DOCUMENT

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

Sex	Frequency	Percent	Cumulative Frequency	Cumulative Percent
?	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

Sex	Frequency	Percent	Cumulative Frequency	Cumulative Percent
?	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

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

Frequency Missing = 1

US EPA ARCHIVE DOCUMENT

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

Sex	Frequency	Percent	Cumulative Frequency	Cumulative 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

Sex	Frequency	Percent	Cumulative Frequency	Cumulative 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

Sex	Frequency	Percent	Cumulative Frequency	Cumulative 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

Sex	Frequency	Percent	Cumulative Frequency	Cumulative 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

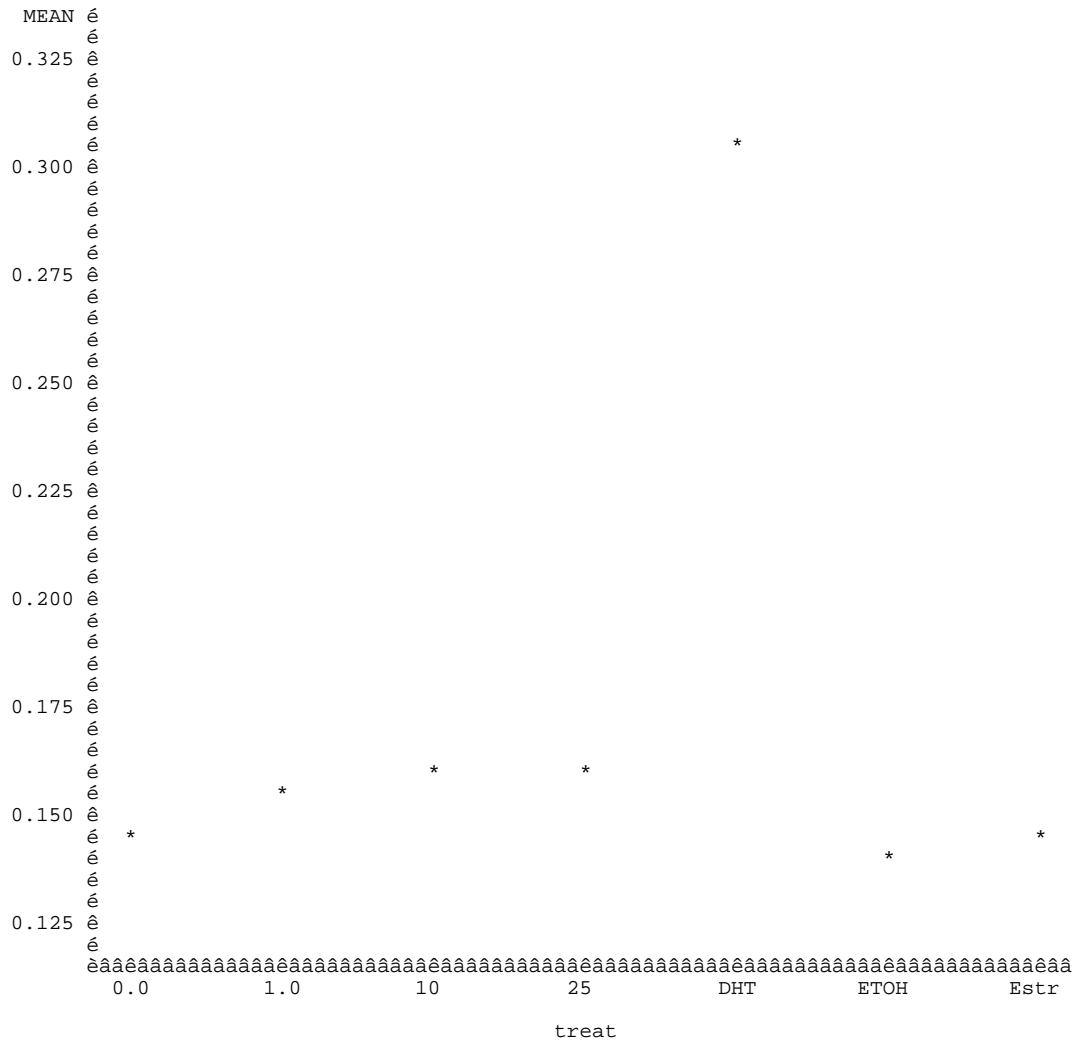
Obs	treat	_TYPE_	_FREQ_	MEAN	STDERR
1	0.0	0	56	0.14694	0.003893
2	1.0	0	51	0.15720	0.005215
3	10	0	55	0.15998	0.003926
4	25	0	54	0.15752	0.004347
5	DHT	0	12	0.30669	0.012958
6	ETOH	0	12	0.14100	0.009301
7	Estr	0	12	0.14480	0.006760

US EPA ARCHIVE DOCUMENT

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 '*'.



US EPA ARCHIVE DOCUMENT

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

Source	DF	Sum of 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

Source	DF	Sum of Squares	Mean 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

Dunnnett'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 Dunnnett's t	2.62465

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

treat Comparison	Difference Between Means	Simultaneous 95% 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

Nonparametric comparison of frog total larynx area over treatments

1231

The NPAR1WAY Procedure

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

treat	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

treat	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
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

US EPA ARCHIVE DOCUMENT

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

Source	DF	Sum of 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

Source	DF	Sum of Squares	Mean 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

Dunnnett'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 Dunnnett's t	2.67093

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

treat Comparison	Difference Between Means	Simultaneous 95% 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

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

Source	DF	Sum of 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

Source	DF	Sum of Squares	Mean 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 ***.

treat Comparison	Difference Between Means	Simultaneous 95% 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

Estr - 0.0 -0.006049 -0.038154 0.026055