Data Evaluation Report on Response of the Amphibian Tadpole (*Xenopus laevis*) to Atrazine During Sexual Differentiation of the Testes

EPA MRID Number: None

Data Requirement:

EPA DP Barcode None

EPA MRID Not Assigned
EPA Guideline Open Literature

Test material: **Purity:** 99%
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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Date Evaluation Completed: 05/01/2003

CITATION: Tavera-Mendoza, L., S. Ruby, P. Brousseau, M. Fournier, D. Cyr and D. Marcogliese. 2001. Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the testis. Environmental Toxicology and Chemistry 21: 527 - 531.

EXECUTIVE SUMMARY:

In an effort to examine the effects of atrazine on gonadal differentiation and reproductive impairments, male African clawed frog larvae (Nieuwkoop-Faber Stage 56) were exposed to mean-measured concentrations of atrazine at 18 µg/L (nominal: 21 µg/L) under static conditions for 48 hours. Animals were fasted during the 48 hours test. Total testicular volume decreased significantly ($p = 0.004$) from 0.026 ± 0.003 mm^3 in controls to 0.01 ± 0.001 m^3 in atrazine-treated tadpoles representing a 57% decrease after 48 hours of exposure. The number of spermatogonial cell nests decreased significantly ($p < 0.001$) from an mean of 242.4 ± 35.7 in controls to 72.9 ± 21.8 in atrazine-exposed tadpoles representing a 70% reduction. The number of nursing cells declined significantly ($p < 0.001$) from a mean of 9.62 ± 0.17 in controls to 2.35 ± 0.36 in atrazine treated tadpoles. Testicular resorption was observed in 70% of the male tadpoles exposed to atrazine relative to controls; failure of full development of the testis (aplasia) was observed in 10% of the testes examined. Histological examination of the pituitary suggested that tissues were actively secreting hormones based on the absence of chromophores.

This study provides useful information on hazard identification and measurement endpoints, such as gonadal abnormalities. However, the study does not provide sufficient information to establish a dose-response relationship because only one concentration of atrazine was tested. Other information which is needed to more fully interpret the significance of this study include: documentation of the measurement endpoints, elaboration on the ecological relevancy of the measurement endpoints, and clarification of the number of exposed animals.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Nonguideline Study
COMPLIANCE: Not conducted under full Good Laboratory Practices

A. MATERIALS:

1. Test Material Atrazine

Description:

Lot No./Batch No. : Not reported

Purity: 99% (Sigma Chemical Co., St. Louis, MO)
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: Niewkoop-Faber stage 54 tadpoles just prior to gonadal differentiation.
Weight at study initiation: (mean and range) not reported
Length at study initiation: (mean and range) not reported

Source: Xenopus 1 (Dexter, MI, USA)

B. STUDY DESIGN:

Objective: To examine the effects of atrazine on gonadal differentiation and reproductive impairments.

1. Experimental Conditions

a) **Range-finding Study**: Exposure concentration based on atrazine residues recently reported in St. Lawrence Rive Valley region of Quebec, CA
b) **Definitive Study**

Table 1 . Experimental Parameters

Parameter	Details
Acclimation: period: conditions: (same as test or not) Feeding: Health: (any mortality observed)	 1 week 100-L glass aquaria containing 15-L dechlorinated Montreal tap water at 21°C on a 12:12 (light:dark) cycle Fed specially prepared tadpole diet (Boreal, St. Catherines, Ontario, CA) twice per week during acclimation
Duration of the test	48 hours starting at NF stage 56
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 static NA NA
Aeration, if any	aerated
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	 glass 100-L 15-L fill volume
Source of dilution water Quality:	City of Montreal (Canada) tap water

Parameter	Details
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 pH = 7.6 not reported not reported not reported not reported not reported not reported not reported not reported 21°C not reported not reported
Number of replicates/groups: negative control: treated ones:	2 replicates per treatment
Number of organisms per replicate /groups:	16 tadpoles/replicate
Biomass loading rate	16 tadpoles/15 L
Test concentrations: nominal:	0 and 21 µg/L
Solvent (type, percentage, if used)	none
Lighting	12 hours dark, 12 hours light
Feeding	no feeding during 48-hr exposure
Recovery of chemical Level of Quantitation Level of Detection	Water samples collected at 0 and 48 hours for quantitation by HPLC/MS
Positive control {if used, indicate the chemical and concentrations}	

Parameter	Details
Other parameters, if any	

2. Observations:

Table 2: Observations

Criteria	Details
Parameters measured including the sublethal effects/toxicity symptoms	
Observation intervals	
Were raw data included?	
Other observations, if any	Gonad-kidney prepared for histological analysis. Brain along with attached pituitary stained to determine if cells in the pituitary were actively secreting hormones. Index of total testicular volume , estimated number of spermatogonial cell nests and nurse cell integrity measured in each testis.

For the three testicular parameters, data were ranked to satisfy the assumption of a normal distribution and then subjected to one-way ANOVA followed by Tukey's test (SPSS10 program; SPSS, Chicago, IL

II. RESULTS and DISCUSSION:

Exposure measured at 0 and 48 hours. Control values contained atrazine at < 0.05 µg/L while the nominal 21 µg/L tank contained atrazine at 18 µg/L. Therefore, mean-measured atrazine concentrations were roughly 87% of nominal.

Total testicular volume decreased significantly ($p = 0.004$) from 0.026 ± 0.003 mm3 in controls to 0.01 ± 0.001 m3 in atrazine-treated tadpoles representing a 57% decrease after 48 hours of exposure. The number of spermatogonial cell nests decreased significantly ($p < 0.001$) from an mean of 242.4 ± 35.7 in controls to 72.9 ± 21.8 in atrazine-exposed tadpoles representing a 70% reduction. The number of nursing cells declined significantly ($p < 0.001$) from a mean of 9.62 ± 0.17 in controls to 2.35 ± 0.36 in atrazine treated tadpoles. Testicular resorption was observed in 70% of the male tadpoles exposed to atrazine relative to controls; failure of full development of the testis (aplasia) was observed in 10% of the testes examined.

Histological sections of the pituitary revealed only undifferentiated chromophobes in both control and treated tadpoles. No evidence was found of chromophobes, indicating that the pituitary was actively secreting hormones.

The study authors conclude that their findings suggest that the developing germ cells in tadpole testes during testicular differentiation are highly sensitive to atrazine and that because these are primary sources of germ cells for the life of the organism, the effects would not be transient and recovery could not occur. The authors suggest that atrazine may disrupt sexual differentiation by altering testosterone metabolism through up-regulation of CYP19 and consequent increased activity of aromatase. Increased aromatase activity could increase transformation of testosterone to estrogen. Alternatively they suggest that atrazine could directly block testosterone and/or dihydrotestosterone at the receptor level. The authors claim that the potential for extrapolating their results to other vertebrates is high given how highly conserved testosterone and estrogen receptor–ligand binding sites are as well as the mRNA nucleotide sequence for both 5α-reductase and aromatase.

This is a nonguideline study and does not provide the level of detail in terms of methodology and results that EPA typically uses to evaluate studies. The methodology section contains some inconsistencies in terms of the number of animals used in the study. This study provides data on the effects of atrazine on testicular development; however, with only a single concentration of atrazine tested, it isn't possible to characterize a dose response. The measurement endpoints are relatively undocumented in *Xenopus laevis* and it is unclear how the effects discussed in this paper may impact reproductive capacity, growth and/or survival of affected animals. Taken at face value, the effects observed in the testes after only 48 hours of exposure are rather remarkable. However, the scope of this study is quite limited and is unrepeated, even within this laboratory.

F. REVIEWER'S COMMENTS:

According to Nieuwkoop-Faber, stage 54 corresponds to age ± 26 days with a length corresponding to 58 - 65 mm.. Stage 56 corresponds to age ±38 days with lengths ranging from 70 - 100 mm.

According to the methods section, there are two replicates per treatment and there are two treatments (0 and 21 µg/L); therefore there are 4 tanks in total with 16 tadpoles per tank. This should yield 64 tadpoles in the study; however the authors claim that the total number of tadpoles for the experiment was 48.

According to the study, the total number of males was 24 and the distribution of males in each of the tanks was 7 and 9 in the controls and 8 and 8 in the treated tanks (7+9+8+8= 32 males) not 24 males.

Study used dechlorinated tap water. EPA recommends the use of dechlorinated water unless chlorine residue analysis is conducted to verify that the water is indeed dechlorinated. Additionally, a limited number (pH and water temperature) of water quality parameters are reported.

Stock solution of atrazine prepared by dissolving 0.15 g atrazine in 1 L distilled water and ultrasonicating for 6 hours in an ice bath. This is relatively harsh treatment for a compound. Why not make up a more dilute solution? Stock solution: 0.1485 mg a.i./mL (21 mL) = 0.3119 mg a.i./15L = 20.79 µg a.i./L treatment solution.

While apparently substantial declines in testicular volume (57%) and spermatogonial cell nests (70%) occurred following exposure to atrazine 18 µg a.i./L and testicular resorption was observed in 70% of the male tadpoles exposed to atrazine relative to controls, no data are provided on the extent to which reproductive success would be impaired. With a single dose of atrazine tested, there is no way to determine whether the effect is dose-related. Also, the methodology section contained some inconsistencies on the actual number of frogs used in the study; the actual sample sizes are not clear. However, in general while sample reported sizes were not high, the treatments were replicated.

Taken at face value, the effects observed in the testes after only 48 hours of exposure are rather remarkable. However, the scope of this study is quite limited and is unreapeated, even within this laboratory. Unfortunately, the study does not include measurements on the gonads of organisms prior to the exposure.

H. REFERENCES:

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

Nieuwkoop, P. D. and J. Faber. 1994. Normal table of *Xenopus laevis* (Daudin). North-Holland Publishing Company, Amsterdam.