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

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: 04/30/2003

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 ovary. Environmental Toxicology and Chemistry 21: 1264 - 1267.

EXECUTIVE SUMMARY:

In an effort to examine the effects of atrazine on gonadal differentiation during larval tadpole development of the female African clawed frog (*Xenopus laevis*), 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-hour test. The frequency of occurrence of primary oogonia was significantly ($p < 0.05$) lower in atrazine-exposed (43.7%) tadpoles relative to controls (74%); however, the frequency of occurrence of secondary oogonia was significantly ($p < 0.05$) higher in atrazine-exposed (36%) tadpoles compared to controls (23%). The incidence of atretic primary and secondary oogonia was significantly higher ($p < 0.05$) in atrazine-exposed ovaries (20.2%) relative to control (2%). Furthermore, sections of the pituitary revealed no histological evidence that the pituitary was actively secreting hormones. The authors concluded that atresia could reduce the reproductive capacity of the tadpole because primary germ cells provide oocytes for all the subsequent cycles of oogenesis in th reproductive life of the frog. Although the authors speculated that atrazine may be affecting aromatase activity, they were unable to provide a mechanism by which conversion of androgens to estrogen could affect the endpoints measured in the study. In addition, there were no data available to indicate an endogenous source of estrogen in the *Xenopus* gonad during this stage of development.

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. This study provides useful data on the effects of atrazine on ovarian development, but it does not establish a dose-response relationship because only one concentration of atrazine was tested. The measurement endpoints are relatively undocumented in *X. laevis,* and it is unclear how the effects discussed in this paper may impact reproductive capacity, growth and/or survival of affected animals even though the authors stated that atresia could reduce the reproductive capacity of the tadpoles. The effects observed in the ovaries after only 48 hours of exposure are significant, but the study has not been repeated.

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*)
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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 during larval tadpole development of the female African clawed frog.

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)	Progeny were collected from 10 malesand 10 females and were acclimated for one 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	 128 mg/L pH = 7.8 90% saturation not reported not reported not reported not reported not reported not reported not reported 21 ± 0.05ºC 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

Parameter	Details
Recovery of chemical Level of Quantitation Level of Detection	Water samples were collected at 0 and 48 hours for atrazine analysis by HPLC/MS (Bodycote Technitrol (Point Claire, PQ, CA). Cyanazine and simazine concentrations were also measured.
Positive control {if used, indicate the chemical and concentrations}	
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 was prepared for histological analysis. Brain along with attached pituitary were stained to determine if cells in the pituitary were actively secreting hormones. Frequency and occurrence of primary and secondary oogonia and atresia (process whereby developing eggs are resorbed in the ovary) were measured in each ovary.

Statistical analyses employed the nonparametric analog of an unpaired t-test, the Mann Whitney U test.

II. RESULTS and DISCUSSION:

Exposure was 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.

The frequency of occurrence of primary oogonia was significantly ($p < 0.05$) lower in atrazine-exposed (43.7%) tadpoles relative to controls (74%); however, the frequency of occurrence of secondary oogonia was significantly ($p < 0.05$) higher in atrazine-exposed (36%) tadpoles compared to controls (23%). The incidence of atretic primary and secondary oogonia was also significantly higher ($p < 0.05$) in atrazine-exposed ovaries (20.2%) relative to controls (2%).

Sections of the pituitary revealed no histological evidence that the pituitary was actively secreting hormones. Only inactive chromophobes were present in the pituitary of both control and treated tadpoles.

The study authors concluded that their findings suggest that exposure of tadpoles to atrazine at 21 µg/L for 48 hrs during sexual differentiation of the ovary resulted in a 20% decline in primary and secondary oogonia through the process of atresia compared to a 2% decline in the controls. Although the formation of secondary oogonia appears to have increased in atrazine-treated females, 20% of those formed underwent resorption before progressing to the primary oocyte stage. Because these primary germ cells provide oocytes for all the subsequent cycles of oogenesis in th reproductive life of the frog, atresia could reduce the reproductive capacity of the tadpole. The authors speculated that the observed effects are probably not a result of an estrogenic effect of atrazine, because an endogenous source of estrogen was not present in the *Xenopus* eggs during this stage of development. Additionally they believed that atrazine does not affect the hypothalmic-pituitary-gonadal axis. The presence of chromophores in the adenohypophysis of the tadpole suggests that the pituitary was inactive at the time of exposure to atrazine. They suggested, however, that atrazine may be acting on aromatase activity, but they did not indicate how the up-regulation of aromatase activity might affect atresia. The authors discussed a similar study (Tavera-Mendoza et al. 2001) which was conducted in their lab, where atrazine treatment increased the rate of testicular resorption and reduced the number of spermatogonial cell nests thus reducing the overall reproductive capacity of the male for the life of the frog. The effects observed in testes could be related to an up-regulation of aromatase activity. The authors claimed that the potential for extrapolating their results to other vertebrates is high because testosterone and estrogen receptor–ligand binding sites are highly conserved as well as the mRNA nucleotide sequence for both 5α-reductase and aromatase.

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.

This 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 were reported.

A stock solution of atrazine was prepared by dissolving 0.15 g atrazine in 1L distilled water and ultrasonicating it for 6 hours in an ice bath. This is relatively harsh treatment for a compound. Stock solution: 0.1485 mg a.i./mL (21 mL) = 0.3119 mg a.i./15L = 20.79 µg a.i./L treatment solution.

Although cyanazine and simazine concentrations were determined, no data were provided on the results of these assessments.

This study provides useful data on the effects of atrazine on ovarian development, but it does not establish a dose-response relationship because only one concentration of atrazine was tested.

The effects observed in the ovaries after only 48 hours of exposure are significant , but has not been repeated.
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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.