Effects of Atrazine on the Reproductive Fitness in the African Clawed Frog

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1. Introduction

Atrazine is one of the most frequently applied triazine herbicides in the United States. Although it has been considered to be benign to most animals, there are several studies that have indicated that it may have significant impact even when present at environmentally realistic levels in temporary breeding habitats of amphibians, especially adjacent to agricultural fields.

In *Xenopus laevis*, a sentinel species used for toxicological assays, exposure during development delayed time to metamorphosis and reduced body size, potentially increasing the risk of predation and reproductive fitness as adults [1]. Exposure during development has also been reported to demasculinize *X. laevis* and reduce testosterone levels in adult males [2], as well as cause an increase in the percentage of females and the development of hermaphrodites and males with multiple testes at levels of atrazine that are environmentally relevant [3]. There also remains the concern that there are potential negative reproductive effects that are not evident by observing morphology alone. Atrazine exposure caused an increased amount of immature and atretic eggs in females as well as decreased testicular volume and sex cells in males [4, 5]. This would most likely lead to a decreased level of reproductive fitness in these organisms. Studies involving atrazine effects on amphibians have focused only on exposure during the critical developmental period in young tadpoles prior to metamorphosis; the potential impact that atrazine may have on adults exposed as tadpoles and/or on adults not previously exposed is still questionable.

The purpose of this study was to assess the different aspects of reproductive fitness of adult *X. Laevis* exposed to atrazine, following a protocol adapted from Fort et al. [6] that is designed to examine contaminant effects on gamete numbers and condition, mating behavior and success, and embryonic viability. This will be the first attempt to ascertain the effects that adult exposure to atrazine may have on reproductive success as well as possible adverse effects on the offspring generation.

2. Materials and Methods

Adult X. laevis were acclimated for two weeks. At the end of the acclimation, males and females were injected with hCG to induce gamete deposition. Individuals of each sex (n = 8) were randomly assigned to 0, 10, 25, 50 or 100 mg/L atrazine in FETAX water for 30 days; another set of animals were maintained separately for later use as breeding partners (n = 40 of both sex). Four frogs of each sex from each treatment were used to determine reproductive fitness measures; the additional four of each sex were euthanized for gonadal measures.

Reproductive Fitness Measures: Animals were induced with hCG as described above and left overnight to amplex. Participation in amplexus was noted. Following fertilization, 75 zygotes were randomly removed from the clutch for tadpole FETAX analysis [7]. An additional 25 tadpoles were removed from the clutch of each treated female to determine tadpole viability until metamorphosis. The remainder of the clutch of eggs was preserved to determine percent fertilization

Gonadal Measures: Non-mated animals were euthanized and the gonadal tissues were surgically removed, weighed and preserved in formalin. Female oocytes were staged by taking two random subsamples of oocytes from each ovary, with stage I representing the most immature oocyte and stage VI the most mature [8].

3. Results and Discussion

There were no significant effects on normalized ovary weights of females in the different atrazine treatments or on average testis weights of males (Table 1). Also, differences in total oocyte mass and proportions of oocytes in different stages and/or necrotic did not occur among atrazine treatments.

Table 1. Mean values (+ 1 SE) for female ovary weights and testis weight (as proportion of total body weight), total oocyte egg mass (g), and proportions of eggs at various stages

Atrazine Conc. (µg/L)	Ovary Weight (%)	Total	< Stage 3	> Stage 3	Necrotic	Testis Weight (%)
0	11.5 ± 0.03	307.2 ± 22.2	67.6 ± 3.4	29.4 ± 2.8	3.0 ± 0.6	0.19 ± 0.01
10	12.1 ± 0.01	503 .0 ± 55.3	76.7 ± 3.7	20.3 ± 3.3	3.0 ± 0.9	0.15 ± 0.01
25	13.7 ± 0.01	466.3 ± 24.1	70.4 ± 3.7	23.6 ± 4.6	6.0 ± 1.0	0.14 ± 0.01
50	12.8 ± 0.02	465.8 ± 83.9	67.3 ± 2.0	25.4 ± 1.3	7.3 ± 2.1	0.14 ± 0.08
100	9.8 ± 0.03	481.3 ± 29.7	68.2 ± 4.7	22.9 ± 4.0	9.0 ± 1.7	0.15 ± 0.02

There were notable trends among treatments. For example, average testis weight was larger among control males (Table 1); average ovary weight was smaller among females in the 100 mg/L atrazine group. A greater proportion of later stage oocytes were evident among females in the control group, and the proportion of necrotic oocytes increased with atrazine concentration.

The percentage of fertilized eggs, while not significantly different among treatment groups for either males or females, was greatest in the male control group. No apparent dose-related trend was noted for females. A greater variety of abnormalities occurred at higher atrazine concentrations (e.g., thoracic adema, malformed faces and abnormal gut coiling). Mean snout-vent length, weight at metamorphosis, and days to metamorphosis of offspring were not related to atrazine concentrations in a dose dependent manner. Among treatment groups, the proportion of males and females were similar and near equality. However, males tended to occur more frequently than females among those whose mothers were maintained in 100 mg/L atrazine. No obvious gonadal abnormalities (e.g., hermaphrodites or multiple testes) were observed for any treatment group.

4. Conclusions

The trends noted in this study warrant further investigation due to the potential negative impact on long-term population viability. For example, greater ovary weights and more advanced oocyte stages among control females may impact population replacement rates. Of particular interest is the possibility that offspring may be affected by the mother's exposure to atrazine, as suggested by the increased numbers and types of malformations among offspring of females in the higher atrazine concentrations.

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6. References

1. Brown Sullivan, K. and K. M. Spence. 2003. Environ. Toxicol. Chem. 22:627-635.
2. Hayes, T. B. et al. 2002. Proc. Nat. Acad. Sci. 99:5476-5480.
3. Cox, C. And K. Brown Sullivan. Abstract. 24th Annual SETAC Meeting. Austin, TX.
4. Tavera-Mendoza, L., et al. 2002. Environ. Toxicol. Chem. 21:527-531.
5. Tavera-Mendoza, L., et al. 2002. Environ. Toxicol. Chem. 21: 1264-1267.
6. Fort, D., et al. 2001. J. Applied Toxicol. 21:41-52.
7. American Society for Testing and Materials. Standard Guide for Conducting the Frog Embryo Teratogenesis Assay.