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# **A Qualitative Meta-analysis Reveals Consistent Effects of Atrazine on Freshwater Fish and Amphibians**

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**Running title:** Atrazine Meta-analysis on Fish and Amphibians

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The authors declare they have no competing financial interests.

**Abbreviations:**

EEC	expected environmental concentration
LOEC	lowest observable effect concentrations
TOF	testicular ovarian follicle
USDA	United States Department of Agriculture

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        Effects on immunity

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Effects of Atrazine on Fish and Amphibian Vitellogenin

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1 OBJECTIVE: The biological effects of the herbicide atrazine on freshwater vertebrates are  
2 highly controversial. In an effort to resolve the controversy, we conducted a qualitative meta-  
3 analysis on the effects of ecologically relevant atrazine concentrations on amphibian and fish  
4 survival, behavior, metamorphic traits, infections, and immune, endocrine, and reproductive  
5 systems.

6 DATA SOURCES: We used published, peer-reviewed research and applied strict quality criteria  
7 for inclusion of studies in the meta-analysis.

8 DATA SYNTHESIS: We found little evidence that atrazine consistently caused direct mortality  
9 of fish or amphibians, but found evidence that it can have indirect and sub-lethal effects. The  
10 relationship between atrazine concentration and timing of amphibian metamorphosis was  
11 regularly non-monotonic, indicating that atrazine can both accelerate and delay metamorphosis.  
12 Atrazine reduced size at or near metamorphosis in 19 of 19 studies. Atrazine elevated amphibian  
13 and fish activity in 12 of 14 studies, reduced anti-predator behaviors in six of seven studies, and  
14 reduced olfactory abilities for fish but not for amphibians. Atrazine was associated with a  
15 reduction in 35 of 43 immune function endpoints and with an increase in 13 of 16 infection  
16 endpoints. Atrazine altered at least one aspect of gonadal morphology in eight of 10 studies, and  
17 consistently affected gonadal function, altering spermatogenesis in two of two studies and sex  
18 hormone concentrations in six of seven studies. Atrazine did not affect vitellogenin in five  
19 studies and only increased aromatase in one of six studies. Effects of atrazine on fish and  
20 amphibian reproductive success, sex ratios, gene frequencies, populations, and communities  
21 remain uncertain.

22 CONCLUSIONS: Although there is much left to learn about the effects of atrazine, we identified  
23 several consistent effects of atrazine that must be weighed against any of its benefits and the  
24 costs and benefits of alternatives to atrazine use.

25

## 26 INTRODUCTION

27 The herbicide atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is the second most  
28 commonly used pesticide in the United States (Kiely et al. 2004), and perhaps the world  
29 (Solomon et al. 1996; van Dijk and Guicherit 1999). It is a photosynthesis inhibitor used to  
30 control certain annual broadleaf weeds, predominantly in corn but also in sorghum, sugarcane,  
31 and other crops and landscaping. The environmental risk posed by atrazine to aquatic systems is  
32 presently being re-evaluated by the US Environmental Protection Agency (USEPA: USEPA  
33 2003, 2007). One of the challenges in evaluating the safety of atrazine has been that its  
34 biological effects are highly controversial, and much of the debate in the literature has been  
35 targeted at its effects on freshwater vertebrates (Hayes 2004; Renner 2004).

36 There have been four reviews on the biological effects of atrazine, all of which were  
37 funded by the corporation that produced or produces this chemical (Giddings et al. 2005; Huber  
38 1993; Solomon et al. 1996; Solomon et al. 2008). However, none of the past reviews used a  
39 meta-analytical approach to identify generalities in responses to atrazine exposure. Meta-  
40 analysis, as paraphrased from the USEPA, is the systematic analysis of studies examining similar  
41 endpoints to draw general conclusions, develop support for hypotheses, and/or produce an  
42 estimate of overall effects. This sort of weight-of-evidence approach would provide directional  
43 hypotheses for future work on atrazine. Furthermore, it would offer invaluable information to  
44 regulatory agencies on general and expected impacts of atrazine on freshwater vertebrates that