ATRAZINE Effects in *Xenopus* Aren't Reproducible

The widely used herbicide does not appear to be an amphibian gender bender, according to new studies.

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New research suggests that the herbicide atrazine does not act as an endocrine disrupter in *Xenopus laevis*, the lab rat of frog species. The results mark the latest development in a fierce debate over the potential environmental and human-health effects of this widely used herbicide and the data used to back opposing claims.

Previous findings by Tyrone Hayes, a comparative endocrinologist at the University of California Berkeley, brought together emerging concerns about amphibian declines, endocrine disrupters, and chemical contamination of the environment. In a series of high-profile papers, Hayes reported that tadpoles exposed to levels of atrazine as low as 0.1 parts per billion (ppb) could develop gonadal abnormalities or become hermaphrodites, apparently because of disruptions in their endocrine systems (*Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5476–5480; *Environ. Health Perspect.* **2003**, *111*, 568–575). He proposed that atrazine-induced production of aromatase, an enzyme that converts testosterone to estrogen in vertebrates (including humans), was feminizing the male frogs. Other researchers reported that atrazine had a range of adverse, endocrine-modulated effects on amphibians. However, studies funded by atrazine manufacturer Syngenta did not find adverse effects. A 2003 U.S. EPA Scientific Advisory Panel (SAP) report found fault with all the studies. But Hayes and the Natural Resources Defense Council still contend that, in its regulatory deliberations on atrazine, EPA is biased in favor of Syngenta, and Hayes now actively campaigns for a ban on the use of atrazine.

The new research, soon to be published in *Aquatic Toxicology,* suggests that for frogs, or at least for *X. laevis*, atrazine's effects are not reproducible. In experiments that closely replicate Hayes's work, endocrinologist Taisen Iguchi at the Okazaki Institute for Integrative Bioscience (Japan) and colleagues used two different groups of *X. laevis*—wild-type, mixed-sex tadpoles and a group of all-male tadpoles. They raised the tadpoles in glass aquaria and exposed them to concentrations of atrazine that ranged from 0.1 to 100 ppb. The scientists found no hermaphrodite frogs; no increase in aromatase as measured by aromatase mRNA induction; and no increase in vitellogenin, another marker of feminization. In

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in vitro experiments with *X. laevis* liver cells, they also found no vitellogenin induced by atrazine. In the mixed-sex experiments, they did see more female frogs as the atrazine dose increased, but the researchers say that this could be due to existing variations in the sex ratio of the wild-type tadpoles at the start of the experiment.

WERNER KLOAS

***X. laevis*—could different subpopulations react differently to atrazine?**

Iguchi and colleagues' experiments "appear to be carefully executed and the data thoughtfully interpreted," says endocrinologist Robert Denver of the University of Michigan. He adds that atrazine could have an effect on sex ratio, but the sample size may be too small to tell. He also notes that the experiment conducted with all-male tadpoles found no feminizing effect at two doses previously reported by Hayes to cause hermaphroditism in *X. laevis*. "No estrogenic actions of atrazine were observed with any of the parameters measured, leading the authors to conclude that the differences in sex ratio could not be caused by an estrogenic effect of atrazine. Overall, this appears to be a sound study that does not support the view that atrazine adversely affects amphibian gonadal development through an estrogenic action," Denver concludes.

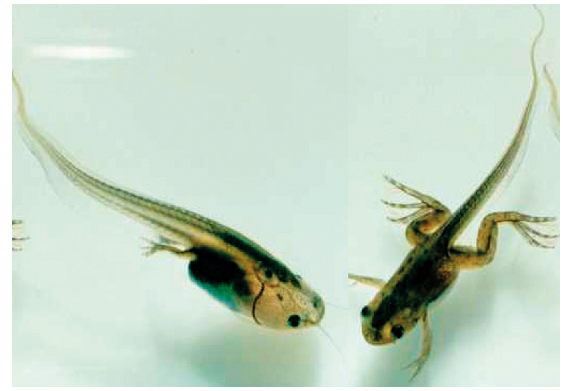
But Hayes disagrees. The increase in female frogs is an important effect consistent with his findings, he says. "You don't know how many females you start with, but when you progressively get a 10% loss in males with every 10 ppb [increase in] atrazine, that is very statistically significant; something is real. Otherwise, each dose should not be statistically different from the controls and should show only slight random variation. [Iguchi and colleagues] did not get effects in their all-male population because they are a different population, and more importantly, they did not test the effective doses with the all-male animals," he adds.

EPA's review

The paper from the researchers in Japan comes in the wake of an EPA SAP review, which concluded in October 2007 that atrazine at environmentally relevant concentrations does not adversely affect the gonadal development of *X. laevis*. The panel evaluated the results of two large Syngenta-funded studies that adhered to an experimental protocol recommended by the SAP in 2003. The studies were conducted by two independent labs—a contract lab, Wildlife International in Easton, Md., and Werner Kloas's lab at the Leibniz Institute of Freshwater Ecology and Inland Fisheries in Berlin. Both labs raised *X. laevis* tadpoles in glass tanks set up with a flow-through water system similar to the system in most fish stores. Five groups, consisting of 200 frogs each, were exposed to 0.01–100 ppb atrazine. The researchers looked for changes in sex ratio and the presence of mixed-sex animals, but they did not find any statistically significant changes.

"The experiments were well run, and for these flow-through systems with this strain exposed to atrazine alone, there was no effect," says David Furlow of the University of California Davis, who is an endocrinologist and is a member of the SAP. In a flow-through system, water moves through tanks so that contaminant concentrations stay relatively constant, but metabolites or biodegradation products do not build up. In static renewal experiments, such as those conducted by Hayes and the group in Japan, metabolites and other chemicals can build up.

Kloas's team also conducted additional static renewal experiments with atrazine concentrations of 25 ppb. They looked for biomarkers of sexual differentiation or for effects on the thyroid system. But they saw no effect on gene expression of sensitive biomarkers in brains and gonads, confirming the results of the Japanese group.



WERNER KLOAS

Three new studies do not see feminizing effects in tadpoles exposed to atrazine during development.

"For me, there is no scientific evidence that atrazine might directly affect gonadal development of *Xenopus*. In general, this [lack of evidence] indicates that there is no direct impact of atrazine on frogs. Thus, the recent results derived from labs in Japan, Easton, and my own in Berlin really undermine the hypothesis that atrazine would directly affect frogs," says Kloas.

Subpopulation differences?

But the SAP stopped short of concluding that the *X. laevis* results could be extended to native North American frogs. It may be the best-studied frog, but "*Xenopus* is like a frog from Mars," says ecologist David Skelly at Yale University. "Using it to evaluate risks to our species doesn't make sense," says Skelly who, like many panel members, says that experiments

should be done on native North American frogs.

Skelly has found high rates of gonadal abnormalities in native frogs in the northeastern U.S. that appear to be related to changes in land use. "This could be exposure to contaminants," he says. But finding the cause of the abnormalities is difficult because "toxicological assays are highly controlled and very specifically tied to individual contaminants. Yet chemical mixtures and a wide variety of other factors could be affecting the frogs." Hayes has reported that mixtures of pesticides at environmental concentrations can adversely affect frogs. In the U.K., where atrazine is not used, biologist Daniel Pickford of Brunel University is also finding an apparent correlation between land use and the incidence of gonadal abnormalities in toads. His preliminary results suggest an additional genetic component to the variation.

Glen Van Der Kraak at the University of Guelph (Canada) also believes that genetics could play a role in frog abnormalities. He has identified three different subpopulations of *X. laevis* in South Africa, to which the frog is native. The research, funded by Syngenta, suggests that these subpopulations vary in their propensity to develop testicular oocytes; this variation may explain some of the conflicting results over the response of *X. laevis* to atrazine, Van Der Kraak told attendees at the Society of Environmental Toxicologists and Chemists annual meeting in November 2007.

Strain differences in response to estrogen, bisphenol A, and dioxin have been reported in rats and mice, and strain difference in response to chemicals has been reported in *Daphnia magna* reproduction tests, notes Osamu Tooi, one of the Japanese researchers. "The discrepancy [between] the present results and Hayes's studies may be caused by various factors, such as genetic differences in *X. laevis* used, water quality, including iodide ions, food, and the composition of aquaria," he says.

But Kloas notes that for *X. laevis*, these differences among subpopulations or strains still need to be proven. "Even in different species of frogs, the response to estrogen under similar conditions is close, within an order of magnitude, so it would be surprising for there to be big subspecies differences. With concerns over feminization, all frog species tested so far react similarly," he says.

Amphibian species are undoubtedly in decline, but the failure of recent studies to find that atrazine feminizes *X. laevis* calls into question the herbicide's role in that decline. "Something or some number of things that we don't understand is going on with frogs," says Skelly.

Rebecca Renner is a contributing editor of ES&T.