

Research, Science & environment

## Pesticide atrazine can turn male frogs into females

*The herbicide atrazine, one of the world's most widely used pesticides, screws up the sex lives of adult male frogs, emasculating three-quarters of them and turning one in 10 into females, according to a new study by UC Berkeley's Tyrone Hayes.*

By [Robert Sanders](#)

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Atrazine, one of the world's most widely used pesticides, wreaks havoc with the sex lives of adult male frogs, emasculating three-quarters of them and turning one in 10 into females, according to a new study by University of California, Berkeley, biologists.

The 75 percent that are chemically castrated are essentially "dead" because of their inability to reproduce in the wild, reports UC Berkeley's Tyrone B. Hayes, professor of integrative biology.

"These male frogs are missing testosterone and all the things that testosterone controls, including sperm. So their fertility is as low as 10 percent in some cases, and that is only if we isolate those animals and pair them with females," he said. "In an environment where they are competing with unexposed animals, they have zero chance of reproducing."

The 10 percent or more that turn from males into females – something not known to occur under natural conditions in amphibians – can successfully mate with male frogs but, because these females are genetically male, all their offspring are male.

"When we grow these guys up, depending on the family, we will get anywhere from 10 to 50 percent females," Hayes said. "In a population, the genetically male females can decrease or wipe out a population just because they skew sex ratios so badly."

Though the experiments were performed on a common laboratory frog, the African clawed frog (*Xenopus laevis*), field studies indicate that atrazine, a potent endocrine disruptor, similarly affects frogs in the wild, and could possibly be one of the causes of amphibian declines around the globe, Hayes said.

Hayes and his UC Berkeley colleagues report their results in this week's online early edition of the journal *Proceedings of the National Academy of Sciences*. In last week's issue of the *Journal of Experimental Biology*, Hayes and colleagues published a review of the possible causes of a worldwide decline in amphibian populations, concluding that atrazine and other hormone-disrupting pollutants are a likely contributor because they affect recruitment of new individuals and make amphibians more susceptible to disease.

"These kinds of problems, like sex-reversing animals skewing sex ratios, are much more dangerous than any chemical that would kill off a population of frogs," he said. "In exposed populations, it looks like there are frogs breeding but, in fact, the population is being very slowly degraded by the introduction of these altered animals."

Some 80 million pounds of the herbicide atrazine are applied annually in the United States on corn and sorghum to control weeds and increase crop yield, but such widespread use also makes atrazine the most common pesticide contaminant of ground and surface water, according to various studies.

More and more research, however, is showing that atrazine interferes with endocrine hormones, such as estrogen and testosterone – in fish, amphibians, birds, reptiles, laboratory rodents and even human cell lines at levels of parts per billion. Recent studies also found a possible link between human birth defects and low birth weight and atrazine exposure in the womb.

As a result of these studies, the Environmental Protection Agency (EPA) is reviewing its regulations on use of the pesticide. Several states are considering banning atrazine, and six class action lawsuits have been filed seeking to eliminate its use. The European Union already bars the use of atrazine.

Hayes's studies in the early 2000s were the first to show that the hormonal effects of atrazine disrupt sexual development in amphibians. Working with the African clawed frog, Hayes and his colleagues showed in 2002 that tadpoles raised in atrazine-contaminated water become hermaphrodites – they develop both female (ovaries) and male (testes) gonads. This occurred at atrazine levels as low as 0.1 parts per billion (ppb), 30 times lower than levels allowed in drinking water by the EPA (3 ppb).

Subsequent studies showed that native leopard frogs (*Rana pipiens*) collected from atrazine-contaminated streams in the Midwest, including from areas up to 1,000 miles from where atrazine is applied, often had eggs in their testes. And many males had lower testosterone levels than normal females and smaller than normal voice boxes, presumably limiting their ability to call mates.

Hayes' research also established that many frogs in Midwestern streams contaminated by atrazine and other pesticides have compromised immune systems, leading to increased mortality from bacterial disease.

Those early studies were hampered by the inability to easily distinguish genetically male from genetically female frogs. Male frogs have two identical sex chromosomes (ZZ) while females have both a Z and a W – the opposite of XX female and XY male humans. But because all frog chromosomes look the same under a light microscope, it's not simple to distinguish male from female.

To overcome this, Hayes' colleague Roger Liu developed a line of all-male frogs so that the genetics would be unequivocal.

“Before, we knew we got fewer males than we should have, and we got hermaphrodites. Now, we have clearly shown that many of these animals are sex-reversed males,” Hayes said. “We have animals that are females, in the sense that they behave like females: They have estrogen, lay eggs, they mate with other males. Atrazine has caused a hormonal imbalance that has made them develop into the wrong sex, in terms of their genetic constitution.”

Coincidentally, another lab in 2008 discovered a sex-linked genetic marker in *Xenopus*, which has allowed Hayes to confirm the genetic sex of his frogs.

In Hayes' study, where 40 frogs lived for about three years after hatching in water with 2.5 ppb atrazine, about 10 percent of the frogs appeared to be resistant to the effects of the pesticide. In ongoing studies, Hayes is investigating whether this apparent resistance is inherited, as well as whether the sex-reversed males have more susceptible offspring.

Syngenta, which manufactures atrazine, disputes many of these studies, including Hayes', that show adverse effects of the pesticide. But Hayes said that “when you have studies all over the world showing problems with atrazine in every vertebrate that has been looked at – fish, frogs, reptiles, birds, mammals – all of them can't be wrong.”

“What people have to realize is that, just as with taking pharmaceuticals, they have to decide whether the benefits outweigh the costs,” he said. “Not every frog or every human will be affected by atrazine, but do you want to take a chance, what with all the other things that we know atrazine does, not just to humans but to rodents and frogs and fish?”

Hayes' long-term studies of the effects of atrazine on frogs have been assisted by many UC Berkeley students, including co-authors on the current paper: undergraduates Vicky Khoury, Anne Narayan, Mariam Nazir, Andrew Park, Lillian

Adame and Elton Chan; and graduate students Travis Brown, Daniel Buchholz, Sherrie Gallipeau and Theresa Stueve.

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