"It's Not Easy Being Green"

Do weed-killers turn frogs into hermaphrodites?

by William Souder

In the summer of 1997, Tyrone Hayes, a biologist at the University of California, Berkeley, accepted what seemed a harmless offer to join a panel of eight other scientists investigating the safety of the common weed-killer atrazine. The panel had been commissioned by atrazine's inventor and primary manufacturer, the Swiss-based chemical giant then called Novartis and since renamed Syngenta. The company wanted to know if its product threatened "non-target" organisms, including fish, reptiles, and amphibians—creatures whose fate had remained largely unexplored through the half century in which atrazine had become the most heavily used herbicide in the United States as well as one of its most widespread environmental contaminants.

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Hayes himself was acutely interested in discovering the causes of a global decline in frog populations that had worried scientists since the early 1990s. Many of the hormones and genes that regulate reproduction and development and metabolism in frogs perform similar functions in people, making frogs important proxies for humans—nature's test animals in a changing world. Syngenta's concern was different. The Environmental Protection Agency had been ordered by Congress to "reregister" atrazine as part of a program to subject a large number of older pesticides to current safety testing, a process that required considerable new data.

Initially, Hayes was asked only to review the scientific literature for studies involving atrazine and frogs. The review turned up nothing, so Hayes designed an experiment to test atrazine directly on the animals. "I honestly thought that the compound wouldn't do anything," Hayes says…

The experiment was similar to ones Hayes had performed many times before. Newly hatched tadpoles were reared in water containing atrazine in amounts ranging from .01 to 25 parts per billion (ppb) until the animals completed metamorphosis. The test animal was the African clawed frog, a species known as the "lab rat of amphibians" and typically referred to by its generic name, *Xenopus.* Once used in human pregnancy testing, *Xenopus* is easier to rear than native North American species, largely because it is entirely aquatic, can be readily force-bred, grows quickly through well-defined stages, and will eat almost any commercial animal feed. Hayes gives his tadpoles Purina Rabbit Chow.

In March 1999, Hayes and his students divided 900 *Xenopus* tadpoles among thirty small aquariums. Half of the tanks contained atrazine; the rest—the control tanks—did not. All the tanks were coded, so neither Hayes nor his students knew which animals were swimming in what dose. Every three days, the tanks were cleaned and the solutions replaced. After forty days, the tadpoles had become frogs. When Hayes examined the frogs, all the control animals were normal. So were all the females. But among the males that had been exposed to atrazine at concentrations of 1 ppb or more, about

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Excerpted from the August 2006 issue of *Harper's Magazine*. The original article goes on to describe how chemical manufacturer Syngenta challenged research it had supported and influenced the U.S. EPA to overlook the dangers of atrazine. William Souder has written *A Plague of Frogs* and was a finalist for the 2005 Pulitzer Prize for his biography of James Audubon.

80 percent had smaller than expected laryngeal dilator muscles—puny voice boxes…

Examining the frogs more closely, Hayes was surprised to discover that about a third of the male frogs exposed to atrazine also had abnormal reproductive organs. Some had malformed or multiple sets of testes. Others had both testes and ovaries, sometimes in odd numbers. The cooccurrence of testes and ovaries is rare in vertebrates and rarer still in *Xenopus.* Yet in Hayes's experiment this morphology had been elicited at concentrations as low as 0.1 ppb, a tenth of the amount that altered their voice boxes. Such a dose is equivalent to a grain of salt dissolved in a ten-gallon aquarium. To put it another way, the federally established "safe" limit for atrazine in human drinking water is 3 ppb, thirty times the dose that turned some of Hayes' frogs into hermaphrodites…

Hayes says that he was naive about how his findings would be received. After reporting his discovery to the other panelists studying atrazine, Hayes argued with them and with Syngenta for months about what to do next. There were protracted discussions about the statistical relevance of the voice-box data and disagreements over the pace of follow-up studies. Hayes was asked for repeated revisions of the "final" report on his results. He saw all of this as an effort to discourage him from publishing his findings. In November 2000 he quit the panel. In his letter of resignation he complained that were he to remain on the team, "recent history suggests that I will spend a great deal of effort preparing reports that will not be finalized in a timely manner, let alone published." He added, "It will appear to my colleagues that I have been part of a plan to bury important data."

…He repeated the *Xenopus* experiment two times, and in April 2002 he published his findings in the *Proceedings of the National Academy of Sciences*…


Dr. Tyrone Hayes

In his published articles, Hayes argued that atrazine activates a gene that produces an enzyme called aromatase, which converts testosterone to estradiol, the strongest of the naturally occurring estrogens. Elevated levels of aromatase, he proposed, could explain the males' stunted voice boxes and multiple, mismatched sex organs—as well as the fact that atrazine appeared to have no effect on the females. Hayes called the process "chemical castration and feminization." He was not surprised that the abnormalities he found were associated with extremely weak doses of atrazine; hormones, including testosterone and estradiol, typically function at very low concentrations. "If you're a toxicologist, this is a low-dose effect," Hayes says. "If you're an endocrinologist, it's a reasonable effect." Chemical poisons tend to be more toxic as the dose increases—the classic "linear" dose-response association. But chemicals that affect hormonal systems sometimes operate in nonlinear ways: In women, for example, estradiol is necessary to stimulate ovulation, but a large dose of estradiol—the amount contained in the birth control pill—cancels this effect.

…According to Bruce Blumberg, an associate professor of developmental and cell biology at the University of California, Irvine, scientists who study endocrine disruption often see dramatic biological effects when they expose cell cultures to weak chemical concentrations. Curiously, Blumberg says, research sponsored by chemical companies rarely detects such effects.

Atrazine is among the world's oldest and most effective herbicides—the aspirin of weed- killers…. Syngenta, a company with roots dating back a couple of centuries that also gave the world DDT and LSD, introduced atrazine to the market in 1959… Syngenta does not divulge sales figures for individual products, but atrazine continues to contribute a significant portion of the company's U.S. revenues from selective herbicides, which last year totaled $1.9 billion worldwide.

Atrazine would still be found in well water for decades even if use of the pesticide were halted today. That very concern led the European Union to ban atrazine in the fall of 2003.

…a fraction of the nearly 80 million pounds of atrazine applied to crops in the United States every year ends up contaminating surface water, groundwater, rain, and even fog…In 2003 the EPA reported that a survey of more than 14,000 water utilities, drawing water from wells in twenty-one states, had found that atrazine, where it previously had been detected, averaged about 0.55 ppb—more than five times the amount that caused abnormalities in Hayes's initial experiment. Because water can take years to percolate down into aquifers, atrazine would still be found in well water for decades even if use of the pesticide were halted today. That very concern led the European Union to ban atrazine in the fall of 2003.

People, unlike frogs, don't undergo critical developmental stages exposed to the elements, and frogs may be particularly sensitive to water-borne chemicals. Still, in the same year atrazine was banned in the European Union, an American epidemiologist named Shanna Swan, then at the University of Missouri School of Medicine, published research showing reduced semen quality in men exposed to pesticides. Swan compared men in Columbia, Missouri, with men living in Minneapolis. The Columbia group had about half as many moving sperm in their semen as their Minneapolis counterparts. Urine samples from the Columbia group showed significantly higher herbicide residues. Swan says few of the men in Columbia were farmers and that she suspects their exposure to pesticides was through drinking water contamination. Reduced semen quality is correlated not only with reduced fertility but also with testicular cancer. One of the pesticides Swan detected in the Missouri group was atrazine.

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…When confronted with evidence that a pesticide has adverse effects, the EPA usually responds with a recommendation that the matter be studied further, and under the peculiar logic of pesticide regulation, it is the manufacturer and not the agency that is responsible for testing chemical products. (The EPA stipulates what kinds of studies are necessary and requires companies to submit raw data in addition to safety conclusions.)

One way to maintain the perception that a pesticide is safe is to take a very long time reviewing information suggesting it is not. The EPA routinely reframes questions about the safety of pesticides in such a way that they remain questions, and evidence of adverse effects usually results in a demand for more study. Pesticide makers are allowed extravagant amounts of time for such follow-up work. And because the companies know the EPA must carefully review every study they submit,

pesticide makers can game the system by submitting flawed and inconclusive research. The EPA then judiciously pores over the new data, finds it wanting, and asks for something more definitive. The oversight the agency thus exercises can be thought of as a kind of business service. The EPA helps chemical companies understand safety concerns in terms of overhead. The agency refers to pesticide makers as "registrants," a term that makes them sound like guests in a luxury hotel, which in some ways does not seem far from accurate.

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In the fall of 2003, the EPA concluded an interim reregistration of atrazine. In compliance with the recommendation of [its] advisory panel, the agency also ordered Syngenta to conduct additional experiments on frogs and atrazine. Two years later, in the summer of 2005, scientists at Syngenta began their initial testing of atrazine on *Xenopus.* They expect to have results by the end of this year, more than four years after Tyrone Hayes proposed the joint experiment that could have resolved the issue in a few months. Meanwhile, in all likelihood, the reregistration of atrazine will be finalized this August.

In January, Hayes published two new papers in *Environmental Health Perspectives.* In one paper, he showed that when frogs are exposed to atrazine in combination with other pesticides—as they are in the environment—the damage to the animals' hormonal systems is more severe than from exposure to atrazine alone. In the other, he reported that when male tadpoles are exposed to estradiol (or to a synthetic compound that suppresses testosterone) they develop the same kinds of gonadal abnormalities that are associated with atrazine—a finding, he argues, that provides further support for his theory of "chemical castration and feminization"…

[Nevertheless, Hayes] knows of no reason why the EPA will not continue to do nothing as the testing moves on to another phase. "My view is that the EPA is never going to take action on atrazine," Hayes says.

Legally, the EPA needn't find a threat to human health to ban atrazine. Adverse effects in the environment are sufficient for the agency to take action, and in the view of many biologists it makes little sense to see humans in isolation from the environment. The question of what direct effects, if any, atrazine has on human health will be hard to answer, and will likely depend on inferences drawn from studies of surrogate species. Such inferences are never certain. Vertebrate toxicology is a kind of Russian roulette: Some species get lucky when they're exposed to chemicals; some don't. Thalidomide—the sedative that caused horrific birth defects in human infants in forty-six countries half a century ago—was believed safe because tests showed it had no effect on rats. In the very same ecosystems where Tyrone Hayes has found abnormal northern leopard frogs, he has also discovered that a close relative of that species—the plains leopard frog—appears to be unaffected by atrazine. As is usually the case with environmental contaminants, the real-world experiment is already up and running.

Photo of Dr. Hayes courtesy of Peg Skorpinski.