

Wreaking Havoc With Life

Minute atrazine levels lead to hermaphroditic frogs, cancer

By Tyrone Hayes

I was a consultant for Novartis and Syngenta, the makers of atrazine. And I learned a lot.

For the past five years, I worked on the widely used herbicide atrazine. In agriculture, it is used on monoculture crops such as corn and sorghum and on stone fruits like cherries. It has been used for forty years, so many of us and many of the environments I talk about have been exposed for many generations. We use more than 76 million pounds annually in the U.S.¹ Atrazine one of the top contaminants of ground and surface waters.² In the U.S and probably in the world, it is the largest selling chemical manufactured by the largest chemical company in the world. It is used on our number one crop in the U.S., corn. And, it is used to fight the most common botanical in the world, a weed called the common groundsel, which has evolved resistance to atrazine in many populations. It has been used in more than 80 countries. Ironically, although we just reregistered it in the U.S., the European Union (EU) banned it two months later. In fact, it has never been used in Switzerland, which is where Syngenta is based.

I am going to talk about a series of studies, most of which have been published.

Then I will get to the new work. My studies are designed to ask control questions in the laboratory about how atrazine impacts development, but also whether our laboratory data mean anything in the wild.

Biological effects of atrazine

What atrazine does is the following. Normally, if you are a male, you should make testosterone. It is testosterone in humans that controls masculine development like deep voice, beard growth and sperm production. Atrazine turns on the enzyme

The following is excerpts from a talk that Tyrone Hayes, Ph.D., professor of integrative biology at the University of California (UC), Berkeley, gave to the 22nd National Pesticide Forum, Unite for Change: New Approaches to Pesticides and Environmental Health, April 2-4, 2004 at UC Berkeley.

aromatase. Aromatase is responsible for converting androgen (a male hormone, such as testosterone) to estrogen.

So amphibians exposed to atrazine suffer two consequences. One, they are demasculinized or chemically castrated because they are losing the androgen. So for example, male frogs voice boxes do not develop. And secondly, they are feminized because now they are making the female hormone, estrogen or estrodial.

The consequences are the following. An African frog exposed to 0.1 parts per billion (ppb) atrazine developed two testes, two ovaries, followed by another testis and two more ovaries.³ This is not a normal animal. The manufacturer argues

that there is background and we are just studying something that naturally occurs. You should not have six gonads and you should certainly not have a mixture of testes and ovaries in your body...even if you are a frog. They are pretty much like humans in that regard.

The next thing I am going to show you is a laboratory animal, a normal, healthy North American frog. I am going to show you that the same type of effect occurs in a North American frog. Under a microscope, a male frog has testicles with testicular tubules. The female has ovaries, with eggs or oocytes that have accumulated in the ovaries. These are normal animals.

The female has ovaries, with eggs or oocytes that have accumulated in the ovaries. These are normal animals.

A North American frog that has been exposed to 0.1 ppb atrazine exhibits two testes, so this frog is not a true hermaphrodite. But, the frog has developed eggs in its testis and the eggs are bursting through the surface of that testis.⁴ That is not normal. So these are two laboratory studies that are very well controlled, so that we can look very specifically at the impact of atrazine.

Effects at minute levels

What I want to do now is give you some perspective. I keep referring to 0.1 ppb. Is this a big or a little number? If you are a visual person, 0.1 ppb or 0.1 micrograms per liter may mean nothing to you. So here is your visual. Imagine a grain of salt. Now divide the weight of that gram of salt by 1000. That is how much atrazine we are adding to these aquaria to produce



the kinds of effects being described. One thousandth of a grain of salt. It is almost nothing.

What I am going to show you now is whether these are what they call "ecologically relevant doses." Atrazine formulations contain 2.9 to 29 parts per million for use on farms. So that is 290 million times what we are using in the laboratory.

Exposure in the danger zone

The published literature shows the range of atrazine in various habitats runoff, temporary pools, permanent water and precipitation. If we look at the "danger zone," the level of atrazine where we saw the effects in the lab (0.1 ppb) up to 10,000 ppb, all of the habitats fall within this zone. This means that there is enough atrazine in rainwater in Nebraska to make hermaphroditic frogs. There is enough atrazine in clouds. There is enough atrazine in snow in the Swiss Alps in Switzerland, where they do not even use atrazine, to make hermaphroditic frogs.

Do effects occur in the field?

When looking at gonads from animals in the field, you can see testes with testicular tubules and nurse cells. But instead of sperm, you will notice it has oocytes (eggs). This is an animal from Wyoming.

Two years ago, the North Platt River in Wyoming was contaminated with atrazine above 0.1 ppb, and 92% of its frogs were hermaphrodites. Exposed animals had three testes filled with eggs. For whatever reason, they did not use atrazine this year and there are zero hermaphrodites. This is an unimaginable experiment. Contaminate an entire river (the contamination comes out of Colorado) and you get these effects. Remove the contamination and it goes away. The company is still arguing that it is just natural variation. If it was natural variation, the effects would be there every year.

Can we blame atrazine?

We have taken these controlled laboratory models where we know it is atrazine. Then we go into the wild and we see these same effects where there is atrazine contamination. The problem is that the laboratory experiments are controlled and the wild data are real, but they are not controlled. So now I am going to show you something that combines the two.

In the spring, they do not just use atrazine. They use five herbicides, two fungicides and three insecticides in Nebraska. In the summer, there are two herbicides left over, atrazine and metolachlor. So the question we next ask is – is it just atrazine or do some of these other compounds in the field cause the problem and what might be the interactive effects of these pesticides.

We tested each one of these chemicals, individually or in combination. Everything is color coded and not labeled by chemical name – because now the industry wants to argue that I am on a mission and I have a bias. The codes are locked in a safe and it is not decoded until we get an answer. In addition, we looked at 3000 frogs, each individually housed and

In EPA's Opinion, How Much Atrazine is OK?

EPA says it is "safe" to be exposed to more than 100 ppb atrazine and allows a limit of 3 ppb atrazine in your drinking water, based on theoretic cancer risks. However, the agency has never specifically tested atrazine at 3 ppb for its health effects. EPA uses large dose studies to determine cancer risks and other health problems, then extrapolates the data to determine the acceptable dosage. This is how they determined that 3 ppb is safe for you to drink. Keep in mind that the effects on amphibians are seen at 0.1 ppb. Pardon the violence in the analogy, but that is like if I shot you with a cannon, and then tried to convince you that the handgun was safe because it was smaller. That is how the decision was made about the safety of your drinking water.

In 1987, EPA declared atrazine a "Possible Human Carcinogen" based on data linking atrazine to cancer in rats. However, after review by the agency's Scientific Advisory Panel, it was downgraded to "Not Likely to be Carcinogenic to Humans" in 2000. The panel said that it was "unlikely that the mechanism by which atrazine induces mammary tumors in female SD rats could be operational in humans." According to Catherine Eiden, a senior scientist in EPA's Health Effects Division, the agency is likely to raise the maximum allowable contaminant level, given the results of recent studies.

To determine whether industry played a role in shaping the EPA's decision, the Natural Resources Defense Council (NRDC) filed a series of Freedom of Information Act requests with the White House and EPA, which failed to produce relevant documents. In November 2003, NRDC filed a lawsuit charging that the White House and EPA were withholding evidence. The following month, the White House released 22 documents, with most of their contents blacked out, including a memo from former Senator Bob Dole to a high-level White House official urging EPA not to restrict atrazine despite the environmental risks. The White House continues to withhold more than 80 other relevant documents.

numbered. This is why I loved Dr. Warren Porter's talk (see *Pesticides and You*, Spring 2004, Vol. 24, No. 1), because we looked at the immune function, growth, development, and problems with the gonads. And we are finding much of the same types of effects in amphibians that you would find in humans – retarded growth, retarded development and gonadal and immune system problems.

When looking at the amount of time it takes frogs to complete metamorphosis in a control group (no pesticide

exposure), a summer group (exposed to two pesticides) and a spring group (exposed to ten pesticides), we see that the more chemicals they are exposed to, the longer it takes to metamorphose. So imagine metamorphosis being like birth. It is controlled by corticoids and thyroid hormone. Think about your size at metamorphosis as birth weight. The longer you are pregnant the bigger your baby should be. The opposite is happening with these animals. The longer they take to metamorphose, the smaller they are. So think of this as delayed delivery with low birth weight.

Thyroid glands

Animals exposed to multiple chemicals develop a goiter, just like humans do. The thyroid gland is being affected. The consequence is that only atrazine causes the hermaphroditism, but when atrazine has all of its little friends along, you are exposed to atrazine longer. So the combination of chemicals is not synergistic in a way that it is causing more hermaphroditism, but they are delaying development so that the atrazine exposure is longer and increasing the effects.

Bringing field exposure to the lab

Finally, we really wanted to bring the field home and put it in controlled conditions. So we literally did that. We collected 10,000 gallons of water from the North Platt River. This involved using the Best Western as temporary storage and going out under the cover of night in camouflage to do these experiments. We used frozen containers in an 18-wheeler to bring it

home. It might seem like little boys designed this experiment, but there is a point to it.

In 2001, we had a contaminated river. We brought back the water, frozen. In 2003, there is no contamination, and we collected that water. So here is what we can do. Using the thawed water, we ask, "Can we make those frogs look like they did two years ago if we put them in that water we saved from two years ago?" So we will really go full circle from lab to field.

People do not get it. When I talk in Nebraska, I am talking about something that comes off of your food. I also work in

Africa in Uganda. I guarantee you, if I told the residents of Nabugabo that the water runoff from crop fields, which is put in containers that is used for all of the drinking and bathing water for their community, makes frogs develop eggs in their testes, I guarantee they would see the connection.

I used to think that there was a connection between environmental health and public health. I no longer think that. They are one and the same.

The breast cancer connection

I used to think that there was a connection between environmental health and public health. I no longer think that. They are one and the same. The people that we have to worry about even more than the "every day people," (the people in this room, the people that I go to school with, the people in the ivory tower) are the farmworkers who are exposed to high levels all of the time.

This is relevant to humans. People often say, 'It is just frogs, so who cares?' Well it does not matter whether you are a frog, a dog, a bat, a cat or a human. The compounds and the genes and the hormones that we are talking about are the same.

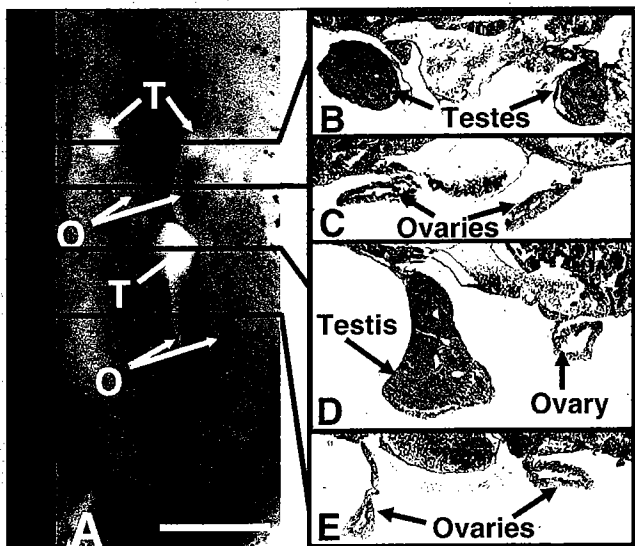


Fig. 1. Gonads of a control postmetamorphic male (A and C) and female (B and D) *X. laevis*. A and B show the entire dissected kidney-adrenal-gonadal complex preserved in Bouin's fixative. C and D show 8 μ m of transverse cross-sections through the animals' right gonad stained with Mallory's trichrome stain. FB, fatbody; K, kidney. Arrows (in A and B) show the anterior and posterior ends of the animals' right gonads. The yellow color in A and B is a result of fixation in Bouin's fixative. Without fixation, the gonad is transparent. The ovary is distinguished by its greater length, lobed structure, and melanin granules. Although some specimens' ovaries lack pigment (especially atrazine-treated animals), testes never have melanin in this species. Histologically, the ovary is distinguished by the ovarian vesicle (hole in the center) along its entire length and the internal ring of connective tissue (in blue). Note the melanin granules (black) in the connective tissue in D.

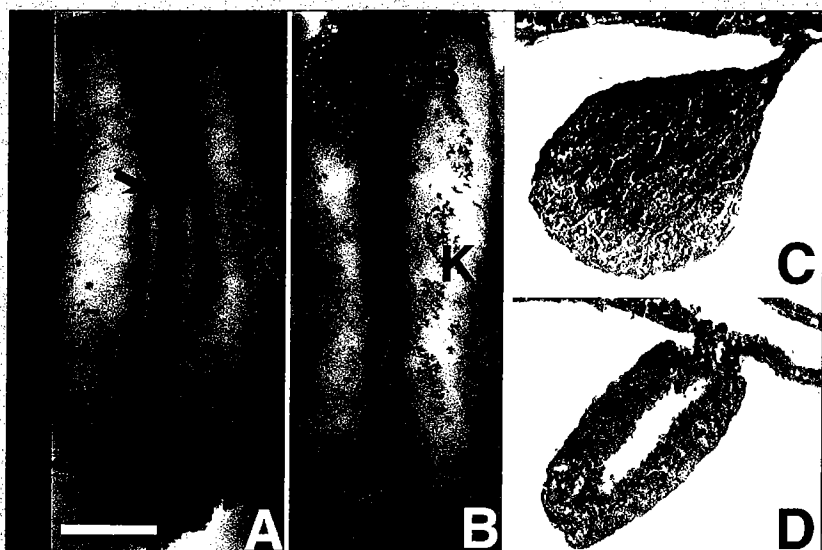


Fig. 2. An atrazine-treated hermaphrodite. The specimen shown was treated with 1 ppb atrazine. A shows the entire dissected kidney-adrenal-gonadal complex. B-E show 8 μ m of transverse cross-sections (stained with Mallory's trichrome stain) through the areas indicated by the lines in A. [Bar = 0.1 mm (A) and 25 μ m (B-E)]. FB, fatbody; K, kidney; O, ovary(ies); T, testis(es). Note the absence of pigment in the ovaries, which was typical of hermaphrodites.

I spend a lot of time in hotel rooms now where they deliver *USA Today*. On the front page the other day was an article about a brand new cancer drug. 40,000 women per year die of cancer, and they have a new drug that is 92% effective at blocking breast cancer return. The drug works the following way. Aromatase converts testosterone to estradiol and estradiol binds to a receptor and causes breast cancer cells to divide. The typical treatment is tamoxifen, which blocks the estrogen receptor. This new breast cancer drug, called exemestane, reduces aromatase, so it reduces the available estrogen to begin with. Now this is crazy, because what atrazine does, and one million people are exposed per day, is just the opposite of our new breast cancer treatment. We know that in humans, it turns on aromatase, promotes estrogen production and breast cancer. So the chemical companies can sell you the dope and the antidote.

Frogs and the human fetus

I have to make one more point. People always ask, why frogs? Well what happens is the following: these tadpoles have the ability to metabolize the pesticides and urinate it out, but they live and drink and reabsorb their urine all the time. We can make this analogy with another aquatic organism, that can also metabolize the pesticides, but they live and drink and reabsorb their urine all of the time – a human fetus.

Recently, I was in Minnesota and I heard someone read a passage that I think expresses this better than I could. The passage is about a woman who just had amniocentesis: "Before it is baby pee, amniotic fluid is water. I drink water and it becomes blood plasma, which suffuses through the amniotic sac and surrounds the baby who also drinks it." An then it goes on to talk about how that water was in creeks and rivers and rains, and the last line of the paragraph is: "Whatever is inside humming bird eggs is inside my womb and whatever is in the world's water is here in my hands." And I thought that just expressed what I was trying to say. And, of course, this book is *Having Faith*, by Sandra Steingraber.



Tyrone Hayes, who received his B.A. in Biology from Harvard and Ph.D. in integrative biology from the University of California (UC) Berkeley, is a specialist in the developmental endocrinology of amphibians, whose work encompasses a "wide sweep in biology." An internationally recognized researcher, he is noteworthy for the large number of undergraduate students who work in his laboratories, co-author papers, and present at professional societies. Dr. Hayes is a tenured professor at UC Berkeley.

Notes

- 1 Environmental Protection Agency. 2003. "Atrazine Interim Reregistration Eligibility Decision (IRED) Q&As." <http://www.epa.gov/pesticides/factsheets/atrazine.htm>.
- 2 Hamilton, Pixie A., et. al. 2004. "Water Quality in the Nation's Streams and Aquifers-Overview of Selected Findings, 1991-2001." U.S. Geological Survey.
- 3 Hayes, Tyrone B. 2002. "Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses." *Proceedings of the National Academy of Sciences*. Vol. 99, No. 8.
- 4 Hayes, Tyrone B. 2003. "Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and Field Evidence." *Environmental Health Perspectives*. Vol. 111, No. 4.