Kermit to Kermette?
Does the Herbicide Atrazine Feminize Male Frogs?

by
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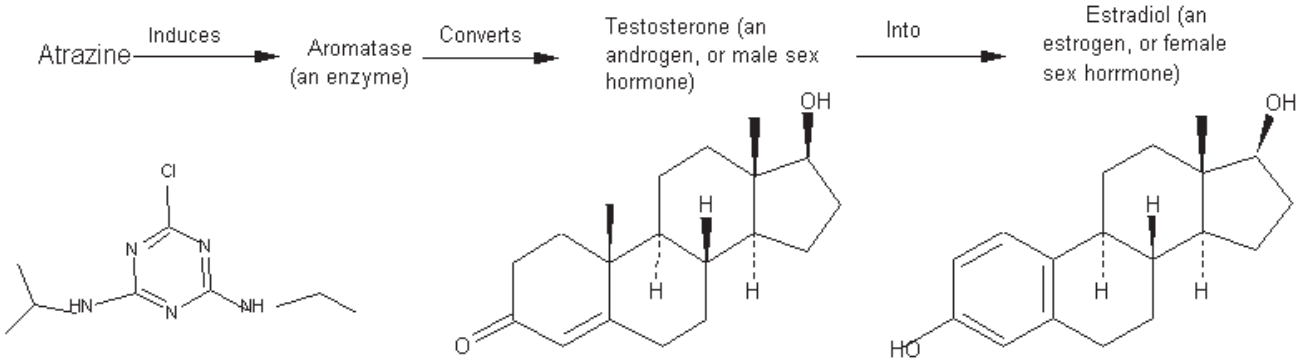
Part I—It's Not Easy Being Green

Atrazine is the most widely used herbicide in the United States. The U.S. Department of Agriculture reports that approximately 70 million pounds were used in the United States in 1995. Not surprisingly, atrazine is a very common contaminant of ground and surface waters. The U.S. Centers for Disease Control's Agency for Toxic Substances reports that when atrazine enters the environment, its life on land is measured in days to months. However, atrazine decomposes only slowly in water, where it can last for much longer times (Agency for Toxic Substances and Disease Registry, 2003). Since the use of atrazine is banned in Switzerland, Denmark, Sweden, Germany, France, and Norway, questions about its safety have inevitably been raised. Earlier studies had shown that atrazine induces (causes) the production of aromatase, an enzyme that converts androgens (male sex hormones) into estrogens (female sex hormones). These studies suggest that the herbicide may act as a chemical castration agent and may be linked to the decline in the population of amphibians that has been observed worldwide. This process and the structures of the chemicals involved in it are shown diagrammatically below:

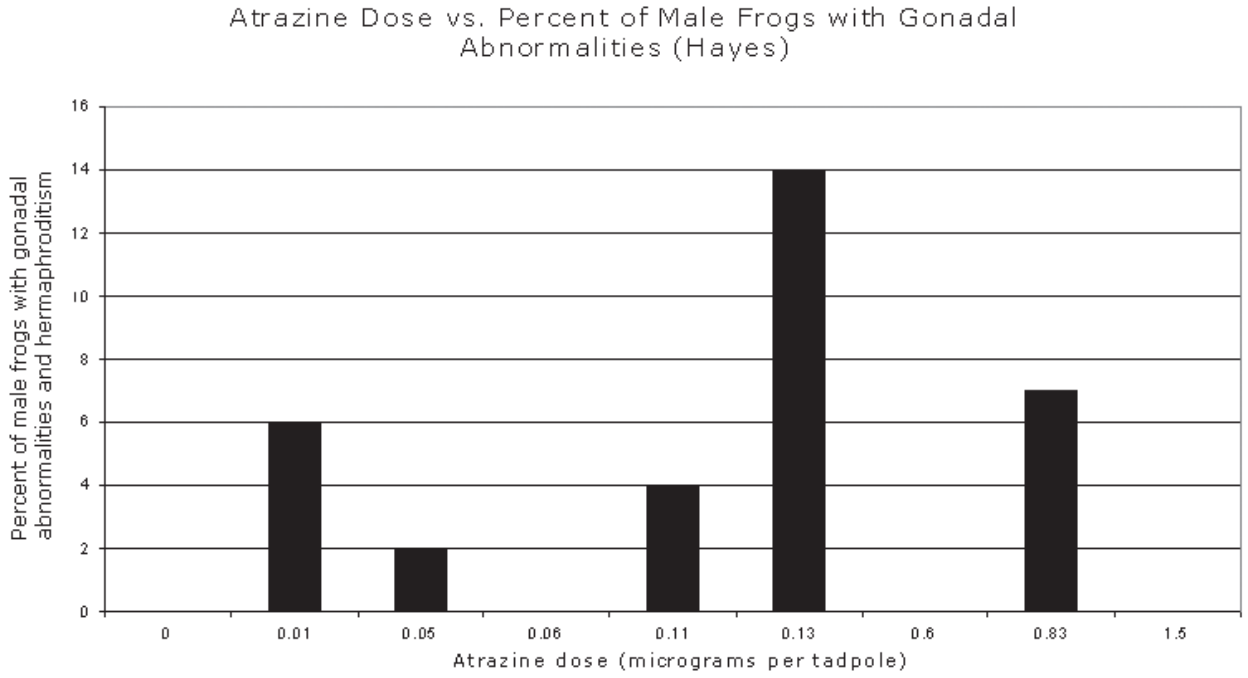


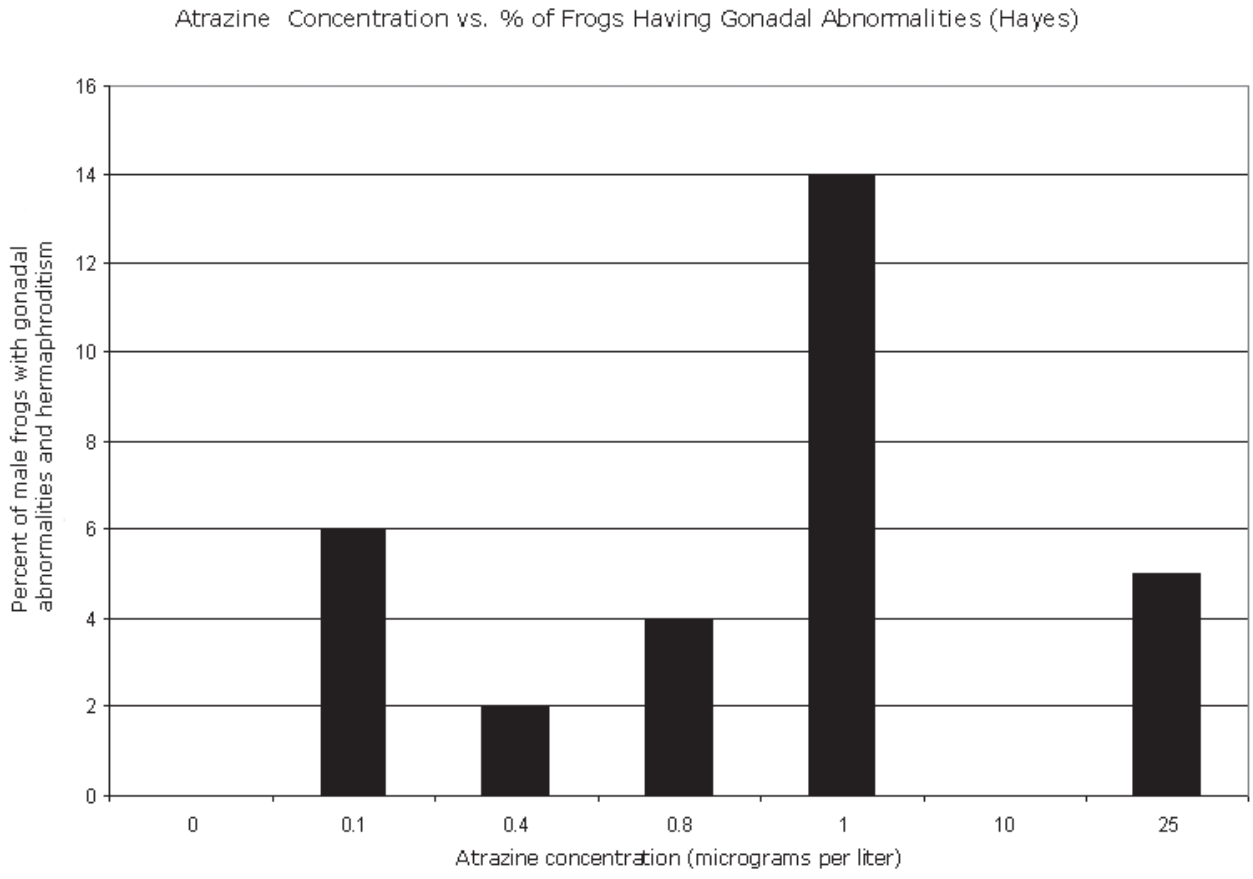
In 1997, the U.S. Environmental Protection Agency (EPA) was in the process of considering the renewal of the Syngenta Corporation's (atrazine's Swiss manufacturer, and one of the world's largest chemical companies) license to continue the use of atrazine in the United States, atrazine's largest market. To provide the needed safety data to the EPA, Syngenta employed Tyrone Hayes, a professor at the University of California at Berkeley, to investigate atrazine's safety.

Professor Hayes began his investigation by growing frog larvae in water samples that he had collected from ponds and streams in areas of the Midwest. Some of these areas used atrazine extensively, whereas others reported little or no use of atrazine. Hayes grew the larvae in water samples that contained a wide range of atrazine concentrations until they developed into sexually mature frogs. Larvae were used since the presence of atrazine could potentially have a significant effect on their sexual maturation and on the biosynthesis of the male sex hormone, testosterone. This hormone must be present in appropriate concentrations for the normal sexual development of male frogs.

Hayes published his findings in articles that appeared in prestigious scientific journals. The data that Hayes gathered show the effect of atrazine on hermaphroditism (the presence of both male and female sexual organs) in male frogs. These data are shown below in graphical form. Note that the second chart compares the concentration of atrazine in the water in which the tadpoles were grown to the percent of gonadal abnormalities found in male frogs, whereas the second chart reports the amount of atrazine to which each tadpole was exposed, the dose. The non-linear nature

of the atrazine concentration and dose ranges reflects the way in which the author reported these data in the original literature (Hayes, 2004). Note that one microgram per liter is equivalent to one part per billion.



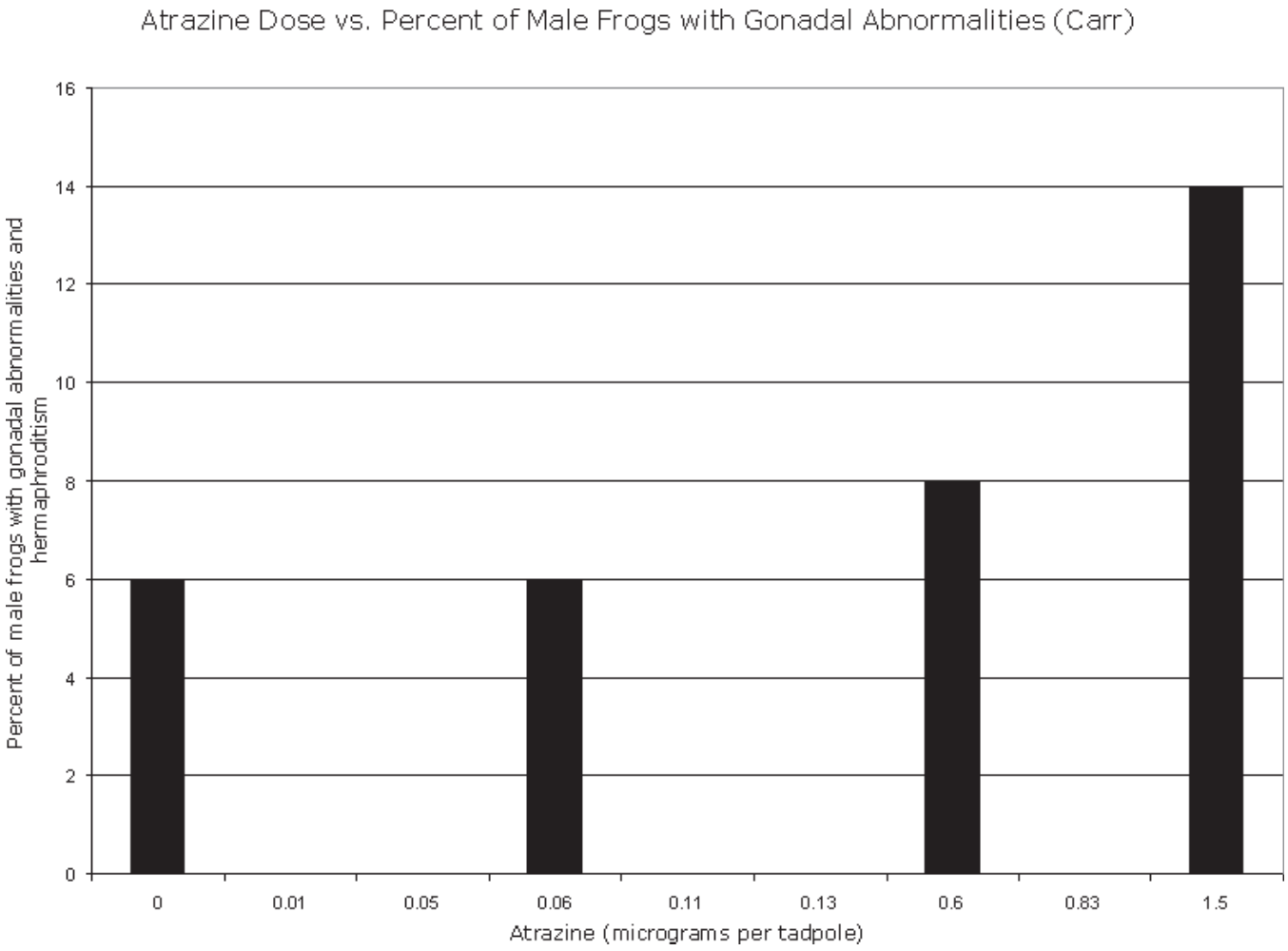


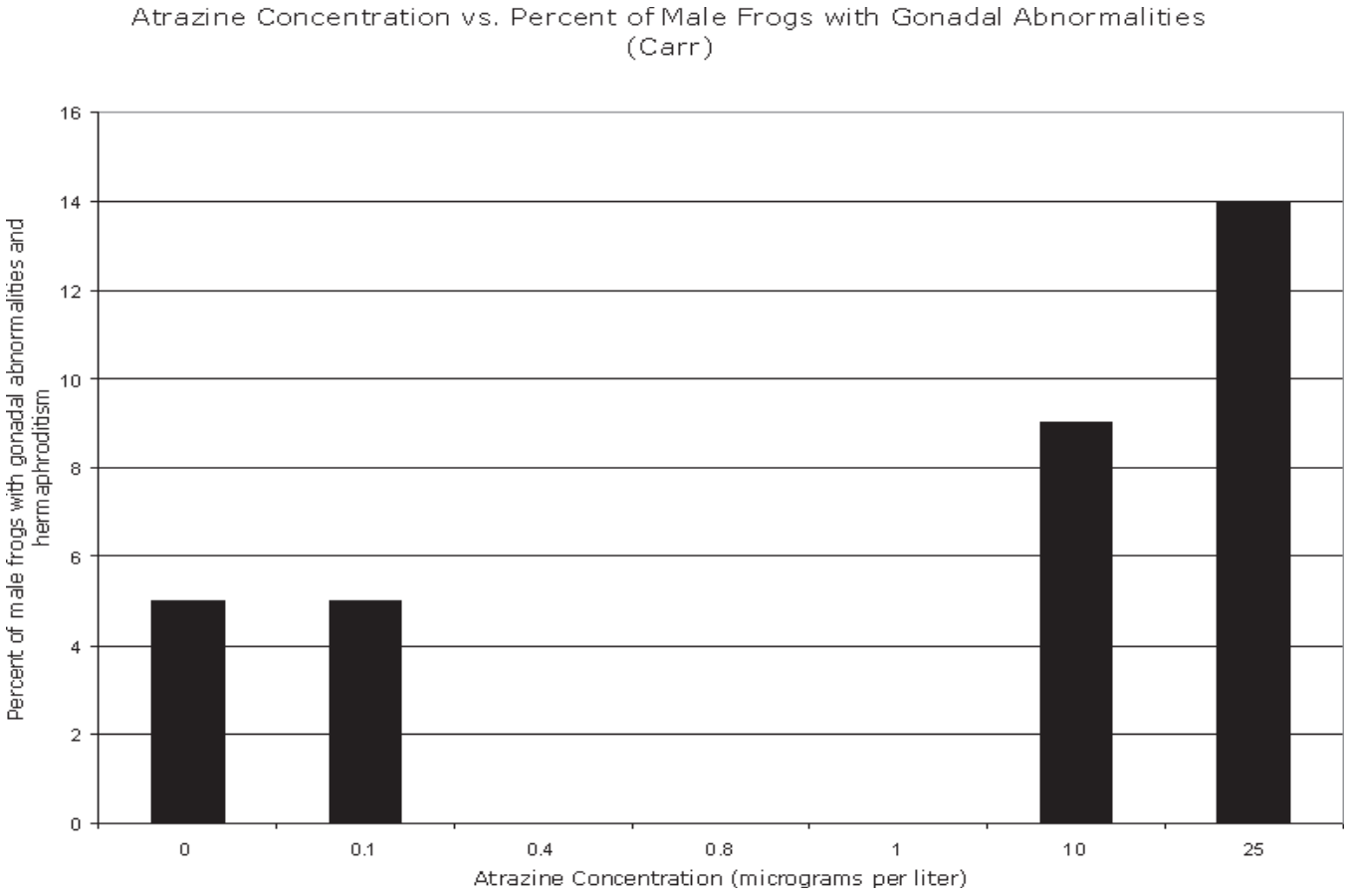
Questions

1. Does atrazine appear to alter male frog development at any concentration?
2. If atrazine does affect male frog development, what is the lowest concentration and dose that appears to have the effect?
3. The chemical DDT was banned for use in the U.S. in the 1960s. For years afterwards, however, American manufacturers of DDT continued to export it to third world countries that had not yet banned its use. How does this observation relate to the use of atrazine in the U.S. today?

Part II—A Different Approach

When Professor Tyrone Hayes submitted his report detailing the outcome of his study to Syngenta, the company rejected his findings and informed him that they would no longer support his work. After terminating its association with Hayes, Syngenta funded new studies of atrazine's safety headed by James Carr, a professor at Texas Tech University, and a team of researchers at Michigan State University (MSU) led by John Gisey, an MSU professor. In Carr's study, frog larvae were exposed to atrazine in nominal (assumed) concentrations of 0, 0.1, 10, and 25 micrograms per liter (ppb) of the herbicide in de-chlorinated laboratory water. The graphs shown below summarize the data reported by Carr's group on the effects of atrazine-induced hermaphroditism, the presence of both male and female gonads, in frogs (Carr, 2003).



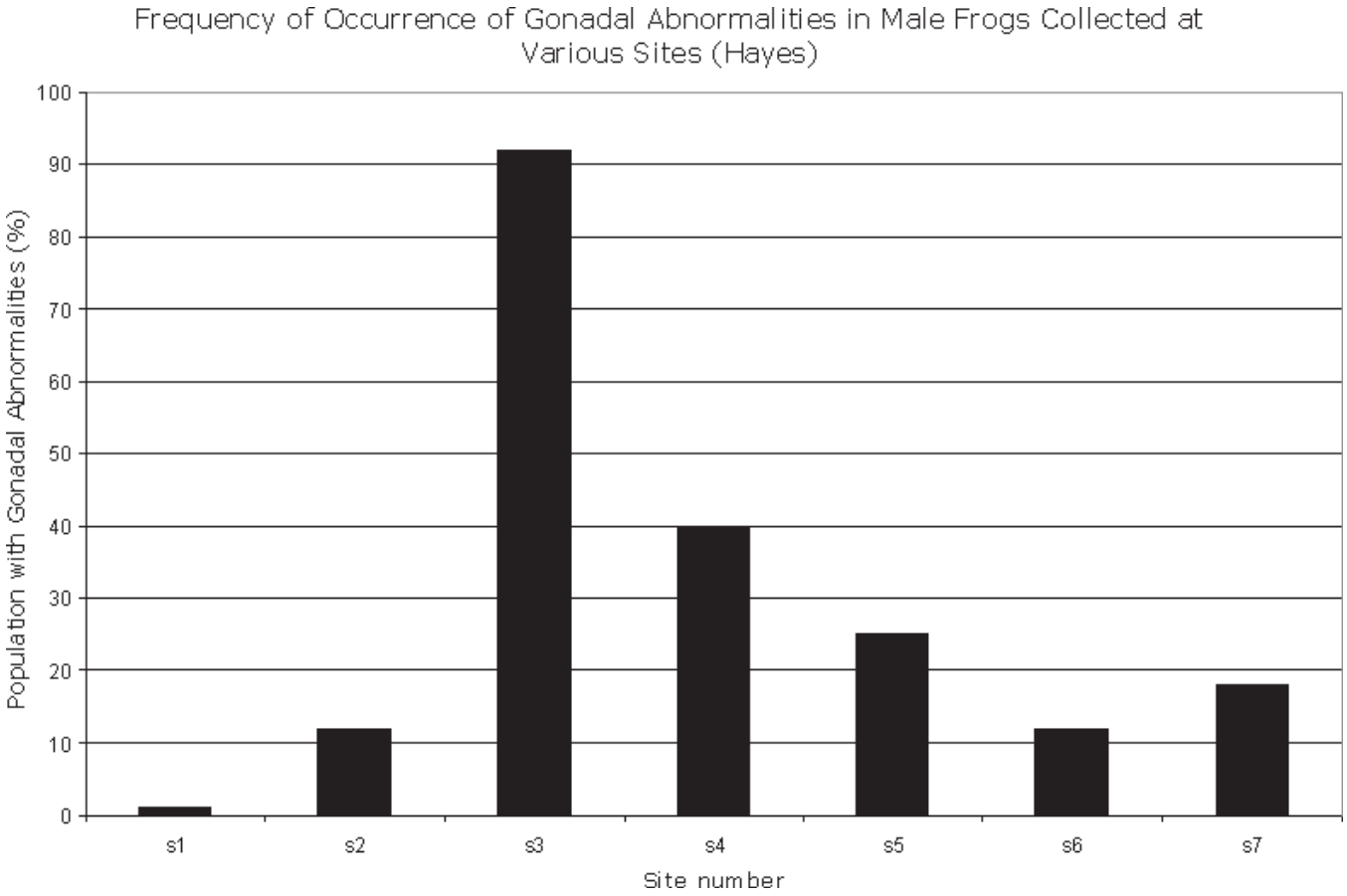


Questions

1. A Syngenta press release quotes James Carr, head of the Texas Tech team, as saying, "We have been unable to reproduce the low-concentration effects of atrazine on amphibians reported elsewhere in the scientific literature." This statement refers to Hayes' results (Hayes, 2002, 2003). Comment on the accuracy of this statement, and explain your reasoning.
2. The Hayes study (Hayes, 2003) was conducted using water samples collected from ponds and streams in agricultural and non-agricultural regions of the Midwest. The study conducted by Carr's group added varying amounts of atrazine to de-chlorinated laboratory water. Which set of experimental conditions, if either, would be more likely to lead to valid experimental results? Explain your reasoning.
3. Comment on the significance of the Carr data, shown above, that reports the percent of male frogs having gonadal abnormalities at a nominal atrazine concentration of *zero* micrograms per liter and a nominal dose of *zero* micrograms per liter.

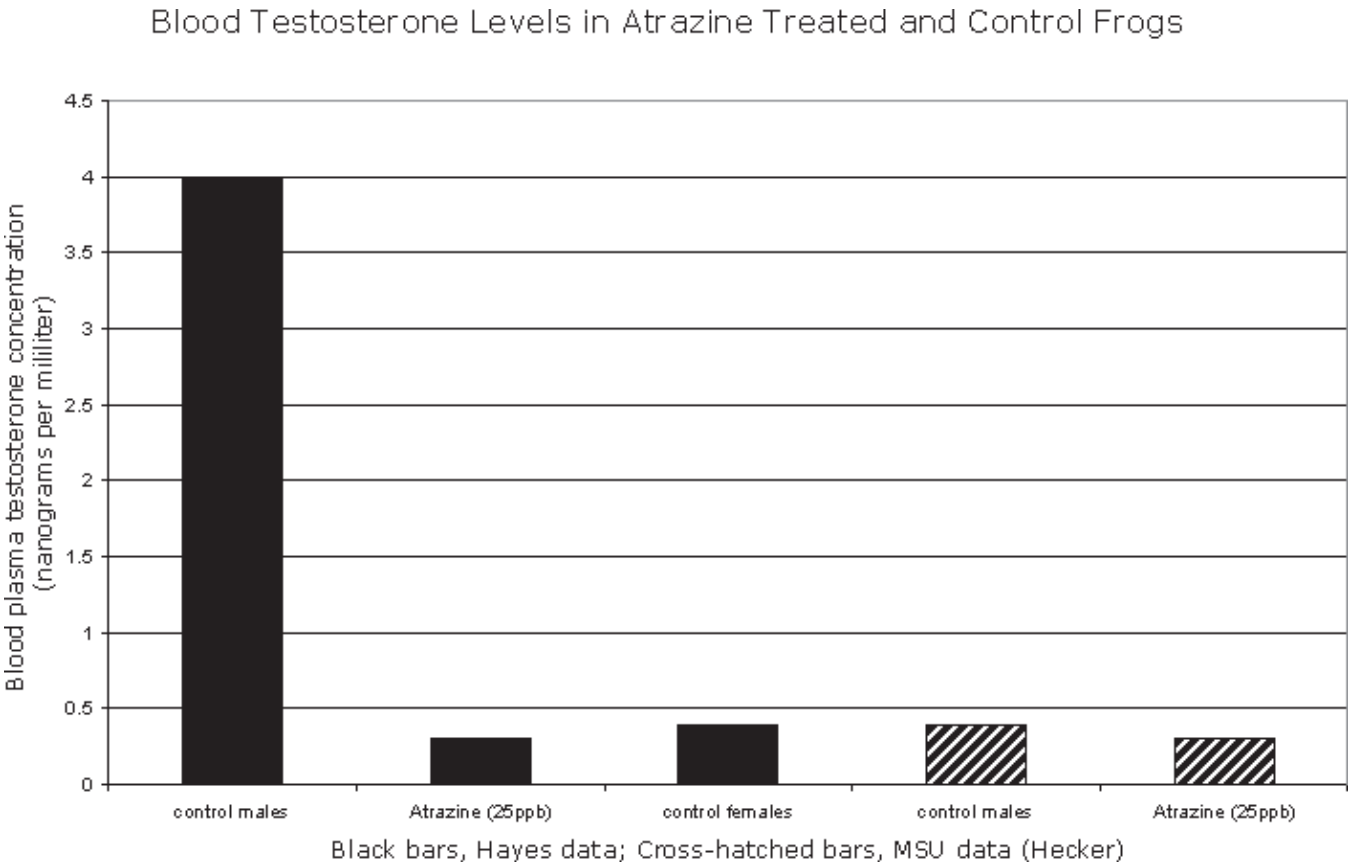
Part III—More from Hayes

Shortly after the work that we considered in Part II of this case study was reported, Hayes published a study in which he used a species of frog *(Rana pipiens)* that he collected in the wild at a variety of sites in the Midwestern U.S. (Hayes, 2003). In this study, Hayes used sales figures for atrazine in the various collection regions to estimate the extent to which the sites were likely to be contaminated with atrazine. The graph shown below compares the percent of frogs having gonadal abnormalities to the experimentally measured atrazine concentration in the water at each site.



Measured atrazine concentration present at each site in parts per billion (micrograms per liter): s1, 0.14; s2, 0.20; s3, 0.20; s4, 0.30; s5, 0.80; s6, 0.70; s7, 0.5

Both Hayes (Hayes, 2003) and a Syngenta supported group (Hecker, 2003) conducted laboratory studies to determine whether the testosterone level in the blood of male frogs was altered by the presence of atrazine in the water in which they grew. In these studies, larvae were grown until they reached sexual maturity in water containing an atrazine concentration of 25 ug/L. For control purposes, both of these studies grew larvae in water containing a nominal (supposed) atrazine concentration of zero ppb. The graph shown below indicates the results obtained in the two studies:



In its evaluation of the data reported in the MSU (Hecker) study, the EPA noted that the controls used in this study were not free of atrazine but were in fact found to contain "atrazine at levels comparable to those in the 0.1 ppb treatment level" (Steeger, 2003). No atrazine was found in the controls used in the Hayes study (Hayes, 2004).

Questions

1. What do the Hayes results indicate about the effect of atrazine on the testosterone concentration in the blood of exposed male frogs?
2. What do the MSU results indicate about the effect of atrazine on the testosterone concentration in the blood of exposed male frogs?
3. Consider the EPA's observation about the presence of atrazine in the controls used in the MSU study. Could this observation explain how Hayes and Hecker could both have accurately reported their experimental observations?
4. In 2003, the EPA recommended that Syngenta's license to continue the use of atrazine in the United States be approved. If one assumes that the data presented here are valid, what factor or combination of factors might have led to this recommendation?
5. Search for two web sites that support the continued use of atrazine and two web sites opposed to its continued use. Prepare an assessment of the validity of these web sites and be prepared to share your observations in class. Useful guidelines for the evaluation of the credibility of web postings may be found at the following URL: **http://www.virtualchase.com/quality/checklist_print.html**.

References

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Steeger, T. M. 2004. Data evaluation report on the response of *Xenopus laevis* to atrazine exposure: assessment of mechanism of action of atrazine. *EPA MRID no. 458677-04 (10 November 2004).* **http://www.epa.gov/oscpmont/sap/2003/june/dataevaluationreports.htm**. Last accessed: 2/17/06.