# Early exposure to common weed killer impairs amphibian development

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Tadpoles develop deformed hearts and impaired kidneys and digestive systems when exposed to the widely used herbicide atrazine in their early stages of life, according to research by Tufts University biologists.

The results present a more comprehensive picture of how this common weed killer – once thought to be harmless to animals -- disrupts growth of vital organs in amphibians during multiple growth periods.

In recent years, worldwide amphibian population declines have fueled concerns over the potentially harmful effects of pesticides on "sentinel" organisms. Previous research had revealed negative effects of atrazine on amphibians extremely early and late in development. The Tufts study, published in the February 2008 edition of "Environmental Health Perspectives," examined tadpoles during an often overlooked period of development, organ morphogenesis.

## Study Results Broadens Knowledge of Herbicide's Effects During a Vulnerable Stage

Organ morphogenesis is a brief, extremely sensitive phase in the tadpoles' growth cycle when they are starting to develop organs, noted Kelly A. McLaughlin, Associate Professor of Biology and lead researcher in the study. She explained that experiments were designed to broaden the understanding of how chemicals affect biological growth in amphibians over multiple stages of development. A $5,000 Tufts

University Faculty Research Marshall Grant helped fund the study.

"Amphibians are very vulnerable to contamination since atrazine is used in the same environs where they live and breed," McLaughlin said.

Atrazine is used to control broadleaf and grassy weeds on golf courses and residential lawns, according to the Federal Environmental Protection Agency. Farmers use it to treat corn and soybeans. Atrazine blocks photosynthesis once it is absorbed by plants. Chronic exposure to the herbicide during metamorphosis altered amphibian gonadal development, according to previous research.

To study the consequences of atrazine exposure during organ morphogenesis, McLaughlin and her colleagues, Professor of Biology J. Michael Reed, doctoral candidate Jenny R. Lenkowski and Lisa Deininger, a Summer Scholars program undergraduate student, collected eggs from adult female frogs and then fertilized them in vitro. Scientists exposed the developing tadpoles to 10, 25 and 35 mg/L of atrazine. The 35 mg/L dosage simulated the average amount of herbicide used when it is applied in the field, said McLaughlin.

## Multiple Impacts

Twelve to 24 hours after exposure to atrazine, tadpoles were examined for abnormal heart growth, visceral hemorrhaging, intestinal coiling, edema and apoptosis (normal cell death that is "programmed" by the body).

Compared with control populations, the tadpoles that were exposed to atrazine had a dramatically higher incidence of abnormalities. The degree of deformities generally corresponded to the size of the dose. After 48 hours of exposure, the point at which organ development is disrupted most profoundly, 57 percent of the tadpoles exposed to 35

mg/L of atrazine had hearts that were smaller than normal, compared with 2% to 3% for the two control groups.

## Ectopic Cell Death

The Tufts scientists also examined atrazine exposed tadpoles for increased incidence of apoptosis by measuring levels of active caspase-3 in the pronephric kidney and midbrain. Caspase-3 is a protein needed for apoptosis to occur. They conducted measurements after 6, 12, 24 and 48 hours of exposure in tadpoles exposed to 25 and 35 mg/l of atrazine. Researchers observed that the atrazine-exposed tadpoles showed significant increases in caspase-3 levels in the kidney and midbrain at 12 hours and beyond when compared with controls. The findings indicated a high incidence of ectopic, or abnormal, apoptosis.

"The increased levels of apoptosis in the midbrain and pronephric kidney we observe suggest that atrazine may cause tissue malformation by inducing ectopic programmed cell death, either directly or indirectly through a mechanism that has not been identified," wrote the researchers.

McLaughlin and her team hope that their findings will lay a foundation for further research to determine the underlying mechanism by which atrazine exposure can affect so many different organ systems during the same stage of early development.

"Our work here documents that atrazine affects amphibian's early development, so the second question is how is this happening?" she said. "We know it blocks photosynthesis in plants but why does it have such negative impact on amphibians?"

Source: Tufts University