

Research suggests EPA pesticide exposure test too short, overlooks long term effects

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The four-day testing period the U.S. Environmental Protection Agency (EPA) commonly uses to determine safe levels of pesticide exposure for humans and animals could fail to account for the toxins' long-term effects, University of Pittsburgh researchers report in the September edition of *Environmental Toxicology and Chemistry*.

The team found that the highly toxic pesticide endosulfan—a neurotoxin banned in several nations but still used extensively in U.S. agriculture—can exhibit a "lag effect" with the fallout from exposure not surfacing until after direct contact has ended. Lead author Devin Jones, a recent Pitt biological sciences graduate, conducted the experiment under Rick Relyea, an associate professor of biological sciences in Pitt's School of Arts and Sciences, with collaboration from Pitt post-doctoral researcher John Hammond. The paper is available on Pitt's Web site at www.pitt.edu/news2009/Endosulfan.pdf

The team exposed nine species of frog and toad tadpoles to endosulfan levels "expected and found in nature" for the EPA's required four-day period, then moved the tadpoles to clean water for an additional four days, Jones reported. Although endosulfan was ultimately toxic to all species, three species of tadpole showed no significant sensitivity to the chemical until after they were transferred to fresh water. Within four days of being moved, up to 97 percent of leopard frog tadpoles perished along with up to 50 percent of spring peeper and American toad tadpoles.

Of most concern, explained Relyea, is that tadpoles and other amphibians are famously sensitive to pollutants and considered an environmental indicator species. The EPA does not require testing on amphibians to determine pesticide safety, but Relyea previously found that endosulfan is 1,000-times more lethal to amphibians than other pesticides. Yet, he said, if the powerful insecticide cannot kill one the world's most susceptible species in four days, then the four-day test period may not adequately gauge the long-term effects on larger, less-sensitive species.

"When a pesticide's toxic effect takes more than four days to appear, it raises serious concerns about making regulatory decisions based on standard four-day tests for any organism," Relyea said. "For most pesticides, we assume that animals will die during the period of exposure, but we do not expect substantial death after the exposure has ended. Even if EPA regulations required testing on amphibians, our research demonstrates that the standard four-day toxicity test would have dramatically underestimated the lethal impact of endosulfan on even this notably sensitive species."

Andrew Blaustein, a professor in Oregon State University's nationally ranked Department of Zoology, who is familiar with the Pitt study, said the results raise concerns about standards for other chemicals and the delayed dangers that might be overlooked. Some of the frog eggs the Pitt team used had been collected by Blaustein's students for an earlier unrelated experiment, but he had no direct role in the current research.

"The results are somewhat alarming because standards for assessing the impacts of contaminants are usually based on short-term studies that may be insufficient in revealing the true impact," Blaustein said. "The implications of this study go beyond a single pesticide and its effect on amphibians. Many other animals and humans may indeed be affected similarly."

Tadpoles in the Pitt project spent four days in 0.5 liters of water containing endosulfan concentrations of 2, 6, 7, 35, 60, and 296 parts-per-billion (ppb), levels consistent with those found in nature. The team cites estimates from Australia—where endosulfan is widely used—that the pesticide can reach 700 ppb when sprayed as close as 10 meters from the ponds amphibians typically call home and 4 ppb when sprayed within 200 meters. The EPA estimates that surface drinking water can have chronic endosulfan levels of 0.5 to 1.5 ppb and acute concentrations of 4.5 to 23.9 ppb.

Leopard frogs, spring peepers, and American toads fared well during the experiment's first four days, but once they were in clean water, the death rate spiked for animals previously exposed to 35 and 60 ppb. Although the other six species did not experience the lag effect, the initial doses of endosulfan were still devastating at very low concentrations. Grey and Pacific tree frogs, Western toads, and Cascades frogs began dying in large numbers from doses as low as 7 ppb, while the same amount killed all green frog and bullfrog tadpoles.

The endosulfan findings build on a 10-year effort by Relyea to understand the potential links between the global decline in amphibians, routine pesticide use, and the possible threat to humans in the future.

A second paper by Relyea and Jones also in the current *Environmental Toxicology and Chemistry* expands on one of Relyea's most notable investigations, a series of findings published in *Ecological Applications* in 2005 indicating that the popular weed-killer Roundup® is "extremely lethal" to amphibians in concentrations found in the environment. The latest work determined the toxicity of Roundup Original Max for a wider group of larval amphibians, including nine frog and toad species and four salamander species. The report is available on Pitt's Web site at www.pitt.edu/news2009/Roundup.pdf

In November 2008, Relyea reported in *Oecologia* that the world's 10 most popular pesticides—which have been detected in nature—combine to create "cocktails of contaminants" that can destroy [amphibian](#) populations, even if the concentration of each individual chemical is within levels considered safe to humans and animals. The mixture killed 99 percent of leopard frog tadpoles and endosulfan alone killed 84 percent.

A month earlier, Relyea published a paper in *Ecological Applications* reporting that gradual amounts of malathion—the most popular insecticide in the United States—too small to directly kill developing leopard frog tadpoles instead sparked a biological chain reaction that deprived them of their primary food source. As a result, nearly half the tadpoles in the experiment did not reach maturity and would have died in nature.

Source: University of Pittsburgh

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