Sublethal Effects of Pesticide Exposure on Amphibian Larvae

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Objectives:

1. To determine the effects of the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) on larval physiology, behavior, and life history of amphibians associated with water features of golf courses.

Start Date: 2001
Project Duration: 2 years
Total Funding: $57,900

Some golf course maintenance practices, especially those that are chemically intensive, may threaten the stability of animal communities. Because of a lack of conspicuous mortality, sublethal effects are likely to go unnoticed during routine population censuses. Sublethal effects compromise the normal functioning of animals on different levels (e.g., physiology, behavior, reproduction) and thus can have substantial population- and community-level effects.

We are concerned with the effects of the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) on larval amphibian physiology, behavior, and life history. We are interested in this compound not just because it has wide application on golf courses, but also because it very water soluble and has a relatively high rate of mobility. It is among the most likely pesticide to be found in golf course wetlands, making it a strong potential threat to amphibian larvae. We have elected to focus on a single model amphibian, the widespread southern leopard frog (*Rana sphenocephala*). This species is one of the leopard frog complex (*R. pipiens* and its relatives), which will give our results broad geographic relevance. The one pesticide-one model approach will allow us to examine the biological and ecological effect of golf course pesticide exposure more thoroughly than in previous studies.

Our previous studies showed that chronic exposure to sublethal concentrations of 2,4-D (Triplet SF Selective Herbicide: 30.56% 2,4-D, 8.17% mecoprop-p, 2.77% dicamba) did not affect larval growth rates nor size at metamorphosis in *R. sphenocephala*. We detected a significant affect on mortality over the 120-day test period that was proportional to the concentration of 2,4-D, and at the highest concentration (285 ppm), no tadpoles survived to metamorphosis.

Researchers at the University of Georgia are investigating the effect of sublethal levels of 2,4-D on developing southern leopard frogs.

Because acute exposure is more likely than chronic exposure, and because acute exposure is less likely to have long-term effects (e.g., physiological or life history effects) we have shifted our attention to the effects of acute exposure on tadpole behavior. These experiments have focused on two variables: locomotor and feeding behavior.

In the locomotor study, we reared 72 *R. sphenocephala* tadpoles in 1.57 L of aged tapwater and fed them *ad libitum* throughout the study period. We exposed and equal number of individuals to three concentration of 2,4-D (285 ppm, 28.5 ppm, and 2.85 ppm) and a control. Within 24 hours, each tadpole was placed in a 2.5-meter swimming chamber (5-cm wide) and prodded at the base of the tail to swim one meter. Each individual was made to swim the 1-meter trial three times with a 20-minute rest between trials. We used a digital video camera to capture the swimming. We determined: 1) the number of swimming burst required to travel one meter, 2) the distance of each burst, and 3) speed of each burst (cm/sec).

We used 36 individuals in the feeding behavior experiment. These individuals were reared as above and then deprived of food for one week prior to observation. Twenty-four hours before observation, an equal number of individuals were exposed to high (285 ppm), low (2.85 ppm), and control (0 ppm) concentrations of 2,4-D. Observation consisted of placing an individual in a 5-liter aquarium that contained either aged tapwater or aged tapwater that previously held sunfish (*Lepomis*). After a brief acclimation period, the tadpoles were then fed. We used a digital video camera to capture the movement and behaviors of the tadpoles over a 30-minute period following feeding. We determined the position of the tadpole within the observation aquarium, the rate of movement, the amount of movement, and the time spent feeding of individuals in predator-free and predator-containing environments. We are still collecting the data from the video tapes in these studies. We hope to have the analyses complete by February, 2004.

The final experiment for this project involves looking at the effects of 2,4-D in semi-natural settings. To accomplish this, we will use cattle tanks as artificial ponds and vary the level of growth (through controlling tadpole density) and 2,4-D exposure (high and low concentration again). We expect 2,4-D to influence the growth of either periphyton or phytoplankton which may have adverse effects on growth and development of the tadpoles. At project's end we will have a more thorough understanding of how chronic exposure to 2,4-D influences amphibian physiology, behavior, and life history (e.g., growth, age/size at metamorphosis).

Summary Points

- While many studies of pesticide exposure use mortality as an endpoint, there may be very serious sublethal effects that are frequently overlooked and thus unaccounted for in experimental studies.
- We focus on effects that alter the normal physiology, behavior, and life history attributes of amphibians. These sublethal effects may go largely unnoticed while they nonetheless affect population viability.
- Our experiments focus on the effects of chronic exposure to 2,4-D on southern leopard frog (*Rana sphenocephala*). We focus on changes in standard metabolic rates, lipid allocation, swimming and feeding behaviors, growth, and age/size at metamorphosis.