## 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.

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The golf course landscape is often a mosaic of habitat types (i.e., lentic and lotic wetlands, forest patches) capable of supporting diverse natural animal communities. Some golf course maintenance practices, especially those that are chemically intensive, may threaten the stability of the communities. Amphibians use aquatic habitats as nuptial/natal sites and tend to be especially sensitive to chemical contaminants. Not all chemical contaminants result in mortality, however, and an appreciation of the sublethal effects of contaminant exposure is necessary to develop a more complete understanding of how maintenance practices influence wildlife communities. Sublethal effects compromise the normal functioning of animals on different levels (e.g., physiology, behavior, reproduction) and thus can have substantial population- and communitylevel 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 (indeed, it has much wider application in other industries), but also because it is 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.

To determine the sublethal effects of 2,4-D on basic physiological processes (growth and metamorphosis), we used 2,4-D in a commonly available commercial form (Triplet SF Selective Herbicide: 2,4-D 30.56%, Mecoprop-p 8.17%, Dicamba 2.77%). We reared 100 *R. sphenocephala* tadpoles for 120 days in chronic exposure trials at four concentrations (control, 285 parts per billion (ppb), 2.85 parts per mil-

lion (ppm), 28.5 ppm, 285 ppm) with static renewal.

The introduction of the pesticide did not affect either the temperature or the pH of the water. At the highest concentrations, there was a significant reduction in dissolved oxygen and an increase in the conductivity. No tadpoles reared at the highest concentration survived past 70 days post-hatch-

ing, and there was a clear linear decrease in survival with increasing 2,4-D concentration. Larval growth rates, however, were unaffected by treatment conditions, nor was size at metamorphosis affected.

Our focus has now shifted to the more novel aspect of the research where we are looking at changes in tadpole behavior, determining the effects of exposure on swimming (sprint speed, endurance) and feeding behavior. The bulk of these data will be collected during winter and early spring 2002-2003. Our preliminary studies have established the repeatability of locomotor behavior in R. sphenocephala and other species of anurans common to golf course wetlands (e.g., Bufo americanus, Hyla versicolor). In addition to looking at the effects on locomotor behavior, we are also conducting experiments designed to determine the impact of acute and chronic exposure on feeding behavior.

## **Summary Points**

□ Our experiments focus on the effects of chronic exposure to 2,4-D on southern leopard frog (*Rana sphenocephala*). We reared tadpoles individually at four concentrations (285 ppb, 2.85 ppm, 285 ppm, 285 ppm) and a control (no 2,4-D).

□ This common herbicide is lethal only after prolonged exposure at relatively high concentrations (e.g., 0% survival to day 70 at 285 ppm, 50% survival to day 70 at 2.85 ppm; no difference from control at 285 ppb).

Growth rate was not affected by pesticide exposure except at the highest concentration.

□ Time to metamorphosis was not affected by pesticide exposure, although the proportion reaching metamorphic climax did decrease with increasing levels of exposure.

□ Current studies are centered on investigating effects of acute exposure at sublethal doses on locomotor and feeding behavior.



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

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