## 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 coures.

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The golf course landscape is often a mosaic of habitat types (e.g., 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. 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 populationand 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 (indeed, it has much wider application in other industries), but also because it very water soluble 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



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

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.

We are using 2,4-D in a commercially available form (Triplet SF Selective Herbicide: 2,4-D 30.56%, Mecoprop-p 8.17%, Dicamba 2.77%). Our pilot studies this summer showed that even high concentrations of 2,4-D did not cause significant levels of mortality in newly hatched gray treefrog tadpoles (*Hyla chrysocelis*). This result indicates that if there are negative impacts of 2,4-D exposure, an analysis of sublethal effects is required.

We are currently running our first set of exposure experiments. Freshly hatched *R*. *sphenocephala* are being raised individually and subjected to chronic 2,4-D exposure during larval development. At regular intervals we are assessing the effects on physiology (standard metabolic rate and lipid allocation) and growth. After metamorphosis, some of the froglets will be tested again to determine if any of the effects carry over to the post-metamorphic phase of the life cycle.

In the upcoming summer (2002), our focus will shift to changes in tadpole behavior,

determining the effects of exposure on swimming (sprint speed, endurance) and feeding behavior. We will use the data collected from the laboratory studies in 2001-2002 to develop a semi-natural mesocosm experiment where biotic effects will be evaluated in conjunction to the exposure effects. 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.

. This research will provide the golf industry with a better understanding of the consequences of improper pesticide application.