

## Multispecies toxicity tests using indigenous organisms: predicting pesticide effects in streams

### Background and goals

Conserving aquatic resources is an important consideration in the development of environmentally sound agricultural practices. Over 1.1 million kilograms, or 2,425,500 pounds, of the insecticide terbufos are used on about 18 percent of Iowa's corn-producing acres annually, creating significant potential for contamination of surface waters. Even when properly applied, such pesticides often enter nearby streams where they can potentially affect the organisms present. Although many such agrichemical contaminants find their way to Iowa's streams and rivers, little research has been conducted to determine their effects on stream organisms.

Managing these hazards to aquatic ecosystems requires predictive techniques that are both cost-effective and accurate. Studies by the U.S. Environmental Protection Agency have demonstrated correlations between response of single species to these chemicals in laboratory tests and field tests. However, such tests cannot predict how much and what kind of damage may occur to the overall ecosystem. *The multispecies toxicity tests undertaken in this project were designed to avoid the cost of underprotection—namely, damage to natural resources—as well as avoid the cost of overprotecting the environment by establishing regulatory requirements that provide no biological benefit*

Multispecies toxicity tests examine a broad range of species that naturally coexist and consider interactions between species. Moreover, they are conducted under environmentally realistic conditions. Although researchers have used ponds to predict pesticide effects on natural communities, they know little

about the sensitivity of stream communities to these inputs, *even though streams are exposed more frequently than lakes and ponds to non-point source agricultural pollutants.*

The objectives of this project were to develop and test a multispecies toxicity testing system capable of predicting the effects of two major corn insecticides, fenvalerate and terbufos, on stream insect communities. Primary emphasis was placed on aquatic insects because (1) they constitute the majority of macroinvertebrate species and individuals in most streams and rivers, and (2) they exhibit a wide range of tolerances to stressors because of their morphological, physiological, and ecological diversity. Insects in this study included mayflies, stoneflies, caddisflies, riffle beetles, and midges.

### Approach and methods

The study was conducted in the Ecosystem Simulation Laboratory at the University of Northern Iowa (UNI). This laboratory is the only one in Iowa, and one of only a few in the country, with capabilities for testing contaminant effects on stream communities. The facility consists of 15 oval, artificial streams, or microcosms, constructed of molded fiberglass (see Fig. 1).

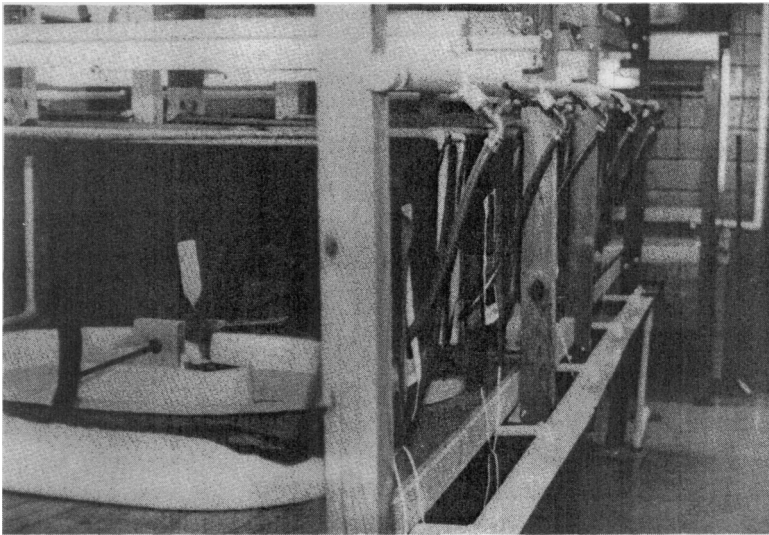
Investigators tested two corn insecticides commonly used in Iowa: fenvalerate, the active ingredient in Pydrin®, and terbufos, the active ingredient in Counter®. Fenvalerate has become popular because of its very low toxicity to humans, and terbufos is the most widely used corn rootworm insecticide in Iowa. In recent years, synthetic pyrethroid insecticides like fenvalerate have taken an increasing share

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### Budget

\$20,000 for year one  
\$24,090 for year two



**Fig. 1. Artificial stream mesocosms in the lab. Each stream channel measures 1.7 by 0.24 by 0.13 meters.**

of the pesticide market because they also control insects effectively while showing low toxicity in birds and mammals. These pyrethroids' popularity and environmental stability increase the possibility of unintentional exposures in aquatic ecosystems where non-target organisms may be adversely affected.

Twelve to thirteen million acres of corn are planted in Iowa each year and approximately six million of these acres are treated for corn rootworm. Terbufos, an organophosphorus insecticide, is widely used in agriculture to control surface and soil feeding insects because it is highly toxic to a variety of target organisms, has low water solubility, and is persistent in a variety of soil types. Previous studies indicate its potential for adversely affecting non-target organisms. Yet, most studies of its effects on non-target insects have used terrestrial rather than aquatic organisms.

Initially, the investigators colonized a food source (periphyton) on foam units in a riffle area along the Volga River in northeastern Iowa. They then transported these units to the laboratory streams in an aerated cooler, where the periphyton developed for five weeks prior to beginning the toxicity tests.

The insects were colonized in the Volga River riffle area in 64 rock-filled plastic containers having six circular holes in each side. The artificial substrates were secured to wooden

frames that had been anchored to the stream bottom with iron rods and concrete blocks. After several weeks, the artificial substrates were transferred to coolers filled with river water and transported to the laboratory, where they were randomly assigned to the 15 artificial streams. The macroinvertebrates were allowed to acclimate for two days before dosing began.

The investigators used five stock solutions of 0.0, 1.0, 10.0, 100.0, and 1000.0  $\mu\text{g/L}$  (parts per billion) active ingredient for both fenvalerate and terbufos. These solutions were replaced every three days, which was especially important for terbufos because of its short half life (the time it takes for half the active ingredient to disappear). The stock solutions were mixed with dilution water to establish concentrations of 0.0, 0.01, 0.1, 1.0, and 10  $\mu\text{g/L}$  in the artificial streams; each concentration was tested in triplicate. Complete replacement of water occurred approximately every three hours. Each stream was covered by an emergence net; overhead lights placed over each stream simulated a period of daylight corresponding to that on day 15 of the test. Stream volume and the circular current in each microcosm were maintained mechanically.

During the test period, investigators sampled water from each microcosm on days 10, 20, and 30 to determine actual insecticide concentrations. In addition, they monitored dissolved oxygen, pH, conductivity, alkalinity, water hardness, temperature, and current velocity to ensure that the stream microcosms reflected those parameters in the natural source ecosystem.

Investigators quantified dead and dying macroinvertebrates (referred to as "drift") by placing a dip net for one minute in the stream one hour after the initial dose. The insects entering the drift were identified, counted, and returned to the microcosm. Emergent adult insects were sampled and preserved every 48 to 72 hours. After 30 days, the contents of each microcosm were washed through a sieve, after which insects were sorted by hand and classified according to size. With the exception of

midges (Chironomidae: Diptera), insects were identified to the lowest possible taxonomic unit using appropriate references, and the species abundances of both adults and immatures were determined for each microcosm.

In determining the abundances of various species, macroinvertebrate taxa that had mean densities greater than or equal to four in at least one treatment were considered a "core" taxon. Total insect densities for each core taxon were compared over all experimental groups to determine concentration effects. Data were analyzed by established statistical procedures.

## Findings

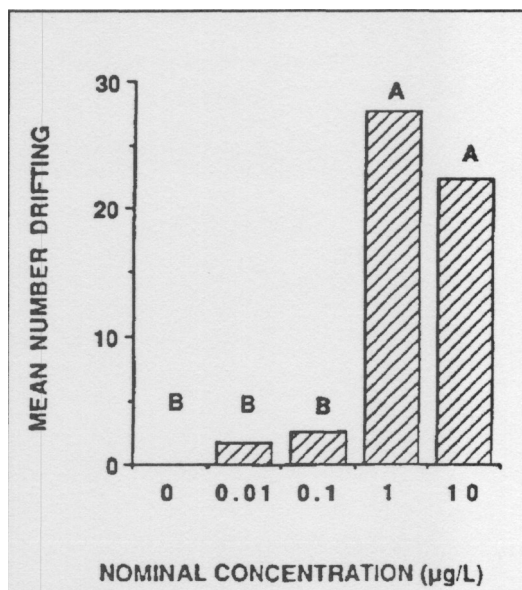
**Fenvalerate:** Because most field exposures are episodic events, the continuous exposures during these 30-day tests subjected riffle insect communities to worst-case conditions. However, drift responses to the initial dose (see Fig. 2) do suggest that short-term exposure to 1.0  $\mu\text{g/L}$  fenvalerate will result in significant impact on riffle insect community structure. (Conversely, it does not appear that short-term exposure to the terbufos concentrations tested severely impacts riffle insects; see terbufos findings below). The insects most frequently entering the drift were mayflies and caddisflies. Under field conditions, fenvalerate may enter aquatic ecosystems through agricultural runoff, aerial drift, or accidental spills. The initial fenvalerate dose in this study, which simulated short-term exposures in the field, indicated that concentrations above 1.0  $\mu\text{g/L}$  will significantly increase drift from the impacted areas.

Analysis of all taxa found during the 30-day test revealed a significant reduction in species richness and total density at 0.1  $\mu\text{g/L}$  fenvalerate. Density reductions were significant in the 0.01  $\mu\text{g/L}$  treatment for one species each of stonefly and larval caddisfly. This finding precluded determination of a community-level NOEC (no observable effect concentration, an individual, standardized concentration limit applied by the U.S. EPA to widely used pesticides). As nominal concentrations increased from 0.01 to 0.1  $\mu\text{g/L}$

fenvalerate, eight core taxa experienced significant density reductions, and the same stonefly species was completely eliminated. With the exception of two caddisfly pupae and a few solitary other larvae remaining in the 10.0  $\mu\text{g/L}$  microcosms, riffle insect communities were completely eliminated as nominal concentrations increased from 1.0 to 10.0  $\mu\text{g/L}$  fenvalerate. In short, this study indicates that aqueous fenvalerate concentrations between 0.01 and 0.1  $\mu\text{g/L}$  will interrupt normal biotic processes in streams and rivers by impacting riffle insect community structure and function.

**Terbufos:** Initial terbufos exposures did not result in significant drift response in any of the concentrations tested. After the 30 days, four taxa, including the most abundant caddisfly, another caddisfly, and two types of midges, exhibited significant mortality at 10  $\mu\text{g/L}$  relative to controls. Species richness and total density were also significantly reduced at 10  $\mu\text{g/L}$  terbufos. However, densities of all taxa in the 1.0  $\mu\text{g/L}$  treatment were similar to those in the control streams.

Overall, the data suggest the following with respect to riffle insect communities: that significant impact occurs at concentrations near 10  $\mu\text{g/L}$ , the NOEC lies above 1.0  $\mu\text{g/L}$ , and terbufos is not as toxic as fenvalerate. There-



*Fig. 2. Mean number of immature aquatic insects from one-minute drift samples taken one hour after the initial fenvalerate pulse dose. Treatments with the same letter are not significantly different.*

fore, if terbufos inputs are regulated to prevent concentrations from exceeding 1.0 µg/L, it should be possible to avoid deleterious effects on riffle insect communities.

### Implications

Pesticides will continue to play a role in sustainable agriculture in Iowa and elsewhere. Still, in our efforts to reduce pesticide inputs, it is important to know how much and what kinds of deleterious effects a given pesticide concentration will cause in various ecosystems. By developing NOECs for widely used pesticides, we can set goals for reducing their input so that the NOEC for a given ecosystem is not exceeded.

For example, this research suggests that NOECs for communities of insects commonly found in Iowa streams lie below 0.01 µg/L fenvalerate and above 1.0 µg/L terbufos. Armed with this information, we should attempt to insure that these NOECs are never reached in Iowa's streams.

Although data are unavailable on fenvalerate and terbufos concentrations in Iowa streams, the suggested application concentrations are several times higher than the concentrations used in these studies. Thus these concentra-

tions are probably environmentally realistic for situations where fenvalerate and terbufos reach stream ecosystems. Further research is needed to monitor actual concentrations of these and other pesticides in Iowa surface waters.

This research has provided a large body of information on pesticide effects on non-target insects living in streams and rivers. These stream insect communities are the most likely aquatic organisms to suffer pesticide exposure, but information on how pesticides affect them has rarely been collected. However, toxicity data from the U.S. Fish and Wildlife Service indicate that Pydrin® is more toxic to fish than Counter®; thus, these insecticides appear to have the same impact in aquatic organisms higher in the food chain. Further research into the effects of widely used pesticides on stream communities should be conducted in order to establish scientifically justifiable NOECs that will protect our aquatic resources.

The results of this project have been presented to a number of local civic and environmental organizations. The Izaak Walton League's national Save our Streams project has also shown strong interest in the data resulting from this project.

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