the same rate over the entire test area. Since less material was used for this type of treatment it may be a more desirable method, especially since this would provide for even less chance for contact or inhalation of the material. A repellent barrier may have several uses, for example, repelling mosquitoes and other insects from a campsite, a picnic area, or various outdoor events.

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TOXICITY OF THE IGR, DIFLUBENZURON, TO FRESHWATER INVERTEBRATES AND FISHES

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ABSTRACT. Technical grade material and wettable powder formulations of the insect growth regulator diflubenzuron and 3 of its degradation products were tested for toxicity to 3 species of aquatic invertebrates and 4 fishes: daphnids (*Daphnia magna*), scuds (*Gammarus pseudolimnaeus*), midges (*Chironomus plumosus*), rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegills (*Lepomis macrochirus*). The acute toxicities of the wettable powder formulation of diflubenzuron ranged from a 48-hr EC_{50} (estimated concentration immobilizing 50% of test organisms) of 0.015 mg/liter for daphnids to a 96-hr LC_{50} (estimated concentration producing 50% mortality) of 660 mg/liter for bluegills. The 96-hr LC_{50} of the technical grade material exceeded 100 mg/liter for all 4 fishes. The most toxic degradation product, 4-chloroaniline, had a 96-hr LC_{50} of 2.4 mg/liter to bluegills and a 48-hr EC_{50} of 43 mg/liter to early fourth-instar midge larvae. The 48-hr $EC_{50's}$ (midge larvae) and 96-hr $LC_{50's}$ for 3 of 4 species of fish for 4-chlorophenyl urea and 2,6-difluorobenzoic acid were greater than 100 mg/liter.

INTRODUCTION

The insect growth regulator, (IGR) diflubenzuron (Dimilin®)[1] is a substituted phenylurea compound that inhibits chitin synthesis during metamorphosis of immature insects (Thompson-Hayward Chemical Company 1974). Diflubenzuron is a member of a group of new chemicals that are proposed as potential alternatives for the more persistent insecticides. These chemicals are indirectly toxic to insects because they interfere with deposition of chitin in the exoskeleton (Wellinga et al. 1973). At molting the larvae are unable to cast their exoskeleton, and either die because the new cuticle ruptures, or from starvation.

Diflubenzuron is biologically active against a variety of target insects, such as mosquitoes (Mulla et al. 1975), house flies (Miller et al. 1975), and alfalfa weevils (Neal 1974). Persistence of this compound in water appears to be limited due to hydrolysis and to adsorption onto organic matter (Schaefer and Dupras 1976). The Environmental Protection Agency (EPA) has granted registration of diflubenzuron for use on the gypsy moth

[1] Reference to the trade name does not imply Government endorsement.

(*Porthetria dispar*), one of the serious defoliators of America's northeastern forests. Experimental permits have been issued to use diflubenzuron in controlling 3 other major insect pests: mosquitoes, the cotton boll weevil (*Anthonomus grandis*), and the range-caterpillar (*Hemileuca oliviae*).

The effect of diflubenzuron on target organisms is well documented. However, information for evaluating its safety to fish and fish food organisms is lacking. Since this compound may be used extensively in both terrestrial and aquatic habitats, it is necessary to determine how it may affect non-target organisms in the aquatic environment. We studied the potential toxicological effects of the parent compound diflubenzuron and three of its known degradation products (Metcalf 1975), to freshwater fish and invertebrates.

MATERIALS AND METHODS

Test animals included: first instar daphnids (*Daphnia magna*), mature scuds (*Gammarus pseudolimnaeus*), early 4th instar larvae of a midge (*Chironomus plumosus*), rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ichtalurus punctatus*), and bluegills (*Lepomis macrochirus*). Daphnids, scuds, and midge larvae were from laboratory cultures. Fingerling fish and rainbow trout eggs were supplied by National fish hatcheries and held in the laboratory according to methods of Brauhn and Schoettger (1975). Average body weights of fingerlings ranged from 0.5 to 2.2 grams.

Technical grade (95%) diflubenzuron and a 25% wettable powder (WP) formulation, and three degradation products (4-chloroaniline, 2,6-difluorobenzoic acid, 4-chlorophenyl urea) were supplied by Thompson-Hayward Chemical Company, Kansas City, Kansas. Technical grade material and the degradation products were dissolved in acetone before addition to the test vessels. The WP formulation was applied directly to the test waters. Chemical concentrations were based on active ingredients.

Acute toxicity tests were conducted in reconstituted water (pH 7.2, alkalinity 35 mg/liter, and hardness 40 mg/liter as $CaCO_3$) at 12 or 22° C and according to standard methods for static toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). The measure of acute toxicity for daphnids and midge larvae was the 48-hr median effective concentration (48-hr EC_{50}) based on immobilization. The susceptibility of other invertebrates and fishes to diflubenzuron was measured in terms of LC_{50}, the calculated concentration of chemical in water which produces a 50% mortality of test organisms during a specific time period. The method of Litchfield and Wilcoxon (1949) was used to estimate the $EC_{50's}$ and $LC_{50's}$ and 95% confidence limits.

Flow-through tests were conducted in well water (pH 7.4, alkalinity 235 mg/liter, hardness 270 mg/liter as $CaCO_3$) at 10°C in a proportional diluter system (Mount and Brungs 1967). Stock solutions of technical grade diflubenzuron were prepared in acetone and delivered to the flow-through system by a metering device described by McAllister et al. (1972).

RESULTS AND DISCUSSION

The acute toxicity of the WP formulation of diflubenzuron varied greatly between species of aquatic invertebrates (Table 1). Daphnids were considerably more sensitive (48-hr EC_{50}, 0.015 mg/liter) than the larvae of midges (48-hr EC_{50}, 0.56 mg/liter). The toxicity of diflubenzuron to midge larvae was determined using the standard 48 hr static test. Results from these tests do not reflect the unique mode of action of IGR's, since a 48 hr exposure is not long enough to induce delayed effects. The WP formulation of diflubenzuron was relatively nontoxic to fish and 96-hr $LC_{50's}$ ranged from 240 μg/liter for rainbow trout to 660 μg/liter for bluegills (Table 1). The 96-hr LC_{50} of the technical grade material ex-

Table 1. Acute toxicity of diflubenzuron (25% wettable powder) to seven species of aquatic organisms in reconstituted water

Organism and stage or weight	Water Temp. (° C)	48-h EC_{50} (daphnids and midges) or 96-h LC_{50}, mg/liter and (95% confidence limits)
Daphnids, 1st instar	22	0.015 (0.010–0.024)
Scuds, mature	12	0.030 (0.019–0.045)
Midge, 4th instar larvae	22	0.560 (0.460–0.680)
Rainbow trout, 1.2 g	12	240 (200–290)
Channel catfish, 2.2 g	22	370 (280–490)
Fathead minnow, 0.87 g	22	430 (360–510)
Bluegills, 0.5 g	22	660 (540–810)

ceeded 100 mg/liter for all fishes. This concentration is 500 times greater than the solubility of technical diflubenzuron in water (Thompson-Hayward Chemical Company 1974).

Toxicities of the 3 degradation products of diflubenzuron against midge larvae and fish covered a wide range, (Table 2). The 4-chloroaniline was the most toxic product to all organisms tested; 96-hr LC_{50}'s ranged from 2.4 mg/liter for bluegills to 23 mg/liter for channel catfish, and the 48-hr EC_{50} for midge larvae was 43 mg/liter. For 4-chlorophenyl urea and 2,6-difluorobenzoic acid, the 48-hr EC_{50} for midge larvae and the 96-hr LC_{50}'s for 3 of the 4 species of fish were all greater than 100 mg/liter.

Eyed eggs and fingerlings of rainbow trout were exposed in a flow-through system for 30 days to concentrations of technical grade diflubenzuron that ranged

Table 2. Acute toxicity of three degradation products of diflubenzuron to five organisms in reconstituted water

Chemical and organism	Water Temp. (° C)	48-h EC_{50} (midge larvae) or 96-h LC_{50} mg/liter and (95% confidence limits)
4-Chlorophenyl urea		
Midge larvae	22	> 100
Rainbow trout	12	72 (57–90)
Fathead minnow	22	> 100
Channel catfish	22	> 100
Bluegill	22	> 100
2-6 Difluorobenzoic acid		
Midge larvae	22	> 100
Fathead minnow	22	69 (55–87)
Rainbow trout	12	> 100
Channel catfish	22	> 100
Bluegill	22	> 100
4-Chloroaniline		
Midge larvae	22	43 (36–51)
Bluegill	22	2.4 (1.8–3.2)
Fathead minnow	22	12 (7–18)
Rainbow trout	12	14 (11–16)
Channel catfish	22	23 (18–29)

from 0.029 to 0.30 mg/liter. These concentrations had no observable adverse effects on the organisms and the rainbow trout eyed eggs hatched and developed normally through sac-fry, swim-up fry, and early fingerling stages. The highest concentration tested was 5 times greater than the maximum recommended concentration for mosquito control (EPA experimental use permit No. 148-EVP-19).

Field studies have shown that diflubenzuron applied to water at a rate of 0.009 mg active ingredient/liter (0.025 lb/acre), which is an effective concentration for controlling larvae of mosquitoes, resulted in temporary reductions of daphnids, mayfly nymphs, and midge larvae (Miura and Takahashi 1975). But other aquatic invertebrates, such as seed shrimp, ostracods, and diving beetles were not affected at a concentration of 0.5 mg/liter (Mulla et al. 1975).

Diflubenzuron is generally applied for mosquito control at rates of 0.02–0.04 lb AI/acre. These applications usually produce initial concentrations of 0.1 and 0.2 mg/liter at depths of 10 cm in natural waters (Schaefer and Dupras 1976). These concentrations are well below those reported in this paper to affect fish populations. However, similar concentrations would be expected to adversely affect populations of daphnids and scuds.

Little is known concerning the accumulation or chronic toxicity of diflubenzuron in aquatic invertebrates after repeated applications. More in-depth studies should be conducted under simulated use—pattern exposures to determine possible effects of this chemical on growth and development of fish food organisms.

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