INVESTIGATIONS IN FISH CONTROL

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UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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Accumulation and Loss of 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73) by Fish: Laboratory Studies

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Abstract

Residues of the 2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73), as determined by gas-liquid chromatography, were rapidly accumulated by fish exposed to the lampricide—in blood plasma, gallbladder bile, and muscle tissue of coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*), and in bile and muscle of channel catfish (*Ictalurus punctatus*) and largemouth bass (*Micropterus salmoides*). Muscle residue levels increased to near the treatment concentration during exposure. After 10 days of withdrawal, residues in plasma, bile, and muscle of rainbow trout and coho salmon decreased to less than 1% of their respective peak concentrations. In all four species, residues in muscle dropped below the limit of detection (0.01 μg/g) within 3 to 14 days. After an initial increase during early withdrawal, bile residues in all species declined steadily but had not dropped below initial levels in channel catfish in 14 days, or below detectable levels (0.01 μg/mL) in coho salmon after 28 days of withdrawal.

The selective lampricide 3-trifluoromethyl-4-nitrophenol (TFM) and the 2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73) are used in combination (98% TFM, 2% Bayer 73) to control sea lampreys (*Petromyzon marinus*) in tributaries of the Great Lakes. Treatment with the combination has been shown to be more effective and less costly than treatment with TFM alone (Howell et al. 1964). Because continued use of the mixture to control sea lampreys is subject to review by regulatory agencies, residue data from fish exposed to the lampricides will be needed to renew registration. Accumulation and loss rates for TFM residues in fish were reported by Sills and Allen (1975), D. C. G. Muir and W. L. Lockhart (unpublished report) determined the rate of uptake of ^{14}C-niclosamide (Bayer 73 without the ethanolamine salt) by fish. In the present study, we exposed four species of fish indigenous to Great Lakes tributaries to Bayer 73 and measured the concentration and persistence of the lampricide in muscle, plasma, and bile.

Materials and Methods

The study was organized in two parts: the determination of the rate of uptake during exposure to Bayer 73 and the determination of the rate of withdrawal or elimination after fish were transferred to lampricide-free water. Two coldwater and two warmwater species of fish were exposed to 0.05 mg/L of technical grade (99.9%) Bayer 73 for 2, 4, 8, 12, and 24 h to determine the rate of uptake of the lampricide. The coldwater species were treated in polyethylene tanks containing 100 L of aerated well water (pH, 8.2; total alkalinity, 85 mg/L as $CaCO_3$; total hardness, 100 mg/L as $CaCO_3$) at 12 ± 1°C and the warmwater species in polyethylene tanks containing 90 L of aerated, limed, spring water (pH, 7.3; total alkalinity, 25 mg/L as $CaCO_3$; total hardness, 23.5 mg/L as $CaCO_3$) at 19 ± 1°C. Coho salmon, *Oncorhynchus kisutch*, and rainbow trout, *Salmo gairdneri* (average weights, 50 and 140 g, respectively), were

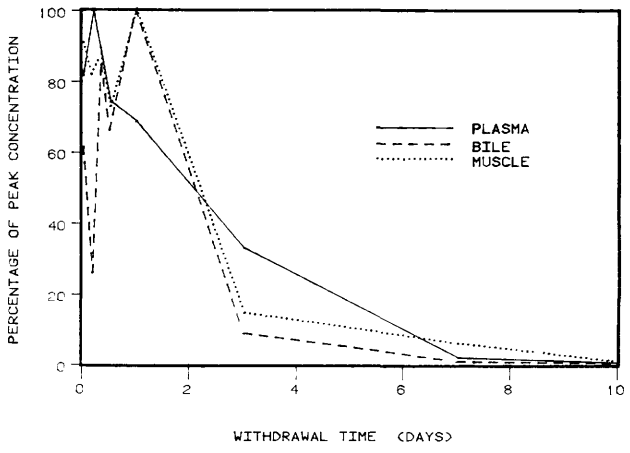

Fig. 1. Accumulation of Bayer 73 residues in plasma, bile, and muscle of rainbow trout during 12 h of exposure to 0.05 mg/L of the lampricide at 12 ± 1°C.

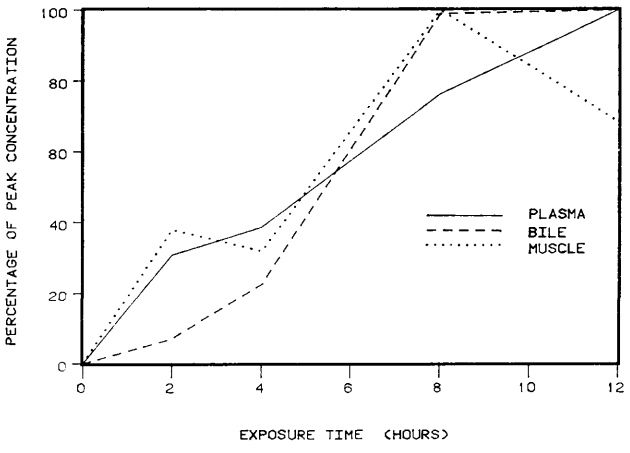

Fig. 2. Loss of Bayer 73 residues in plasma, bile, and muscle of rainbow trout exposed to 0.05 mg/L of the lampricide for 12 h and then transferred to lampricide-free water at 12 ± 1°C.

treated and sampled at the National Fishery Research Laboratory, La Crosse, Wisconsin, and channel catfish, *Ictalurus punctatus*, and largemouth bass, *Micropterus salmoides* (average weights, 280 and 330 g, respectively), at the Southeastern Fish Control Laboratory, Warm Springs, Georgia.

Five fish were exposed in each polyethylene tank. Samples of plasma, bile, and muscle tissue were taken from five rainbow trout at each sampling period in both the uptake and withdrawal studies. Muscle tissue was sampled for largemouth bass and channel catfish during the uptake study and muscle and bile samples were taken from these two species and coho salmon during the withdrawal study in an attempt to identify species differences. Withdrawal studies were conducted with fish that had been exposed to 0.05 mg/L of Bayer 73 for 12 or 24 h, then transferred to lampricide-free water and sampled at selected time intervals thereafter. Blood samples were drawn from the trout and salmon by puncture of the caudal peduncle. In collection of gallbladder bile, fish were stunned by a blow to the head, a midventral incision was made, and bile was removed with needle and syringe.

Fish were not fed during exposure to the chemical, but food was provided after 24 h of withdrawal in fresh water. Because fish were fed during the withdrawal study, the volume of bile available for sampling was relatively small. Therefore the bile from the five fish in each sample group was pooled for analysis.

Frozen muscle samples were prepared for analysis by the method of Benville and Tindle (1970) and extracted by the column technique of Hesselberg and Johnson (1972). Extracts were processed and analyzed by the gas-liquid chromatographic method of Luhning et al. (1979). Blood plasma and bile samples were subjected to alkaline hydrolysis in the presence of hydrogen peroxide and analyzed for Bayer 73 residues by gas-liquid chromatography (Luhning et al. 1979).

Results

Uptake Studies

Fish exposed to Bayer 73 rapidly accumulated the chemical, as demonstrated, for example, in rainbow trout (Fig. 1). Residues in muscle did not continue to increase with increasing exposure time, but rather plateaued and sometimes declined. The maximum residue concentration in muscle tissue of all species exposed was approximately that in the exposure solution (Tables 1 and 2).

The highest residue concentration observed in plasma of rainbow trout was 7.66 μg/mL after 12 h of exposure, whereas residues in bile continued to increase throughout the uptake periods, to 473 μg/mL at 24 h (Table 1).

Withdrawal Studies

Fish exposed to 0.05 mg/L of Bayer 73 for 12 or 24 h and then transferred to lampricide-free water eliminated residues accumulated during exposure. Residues in plasma, bile, and muscle of rainbow trout decreased to less than 1% of their respective peak concentrations within 10 days of withdrawal (Fig. 2). In muscle tissue, residues declined to less than detectable levels (<0.01 μg/g) within 7 days in coho salmon, 10 days in rainbow trout, 3 days in channel catfish, and 14 days in largemouth bass (Tables 3–5). Elimination of residues in plasma to nondetectable levels required slightly longer—14 days in coho salmon and 21 days in rainbow trout (Tables 3 and 4).

Table 1. *Mean concentrations of Bayer 73 in plasma and bile (μg/mL), and muscle (μg/g) from rainbow trout exposed to 0.05 mg/L of Bayer 73 for selected exposure times at 12 ± 1°C (SE in parentheses).*

Exposure time (h)[a]	Plasma	Bile	Muscle
Control	<0.01	<0.01	<0.01
2	2.36	26.6	0.025
	(0.192)	(10.9)	(0.008)
4	2.96	85.4	0.021
	(0.178)	(17.7)	(0.003)
8	5.84	376.0	0.066
	(0.528)	(81.4)	(0.004)
12	7.66	380.0	0.045
	(0.814)	(50.1)	(0.008)
24	5.30	473.0	0.024
	(0.483)	(36.7)	(0.004)

[a]Five fish were sampled at each interval.

Table 2. *Mean concentration of Bayer 73 (μg/g) in muscle tissue from channel catfish and largemouth bass exposed to 0.05 mg/L of Bayer 73 for selected exposure times at 19 ± 1°C (SE in parentheses).*

Exposure time (h)[a]	Channel catfish	Largemouth bass
Control	<0.01	<0.01
2	0.051	0.043
	(0.005)	(0.010)
4	0.034	0.053
	(0.004)	(0.008)
8	0.025	0.048
	(0.001)	(0.007)
12	0.022	0.058
	(0.002)	(0.003)
24	0.019	0.048
	(0.001)	(0.003)

[a]Five fish were sampled at each interval.

Residues in bile continued to increase for 12 to 24 h in coho salmon, rainbow trout, and channel catfish after the fish were transferred to fresh water, before they began trending downward. The rate of elimination of residues from bile was similar to that in plasma and muscle in rainbow trout (Fig. 2), but because the peak concentrations in

Table 3. *Mean concentrations of Bayer 73 in plasma and bile (μg/mL) and muscle (μg/g) from coho salmon exposed to 0.05 mg/L of Bayer 73 for 12 h and transferred to lampricide-free water at 12 ± 1°C for selected withdrawal times (SE in parentheses).*

Withdrawal time[a]	Plasma	Bile[b]	Muscle
Control	<0.01	<0.01	<0.01
Hours			
0	5.00	859	0.101
	(1.89)		(0.006)
4	8.48	892	0.064
	(1.23)		(0.005)
8	6.24	1,240	0.070
	(0.613)		(0.008)
12	6.57	1,260	0.146
	(0.631)		(0.031)
24	5.47	862	0.080
	(0.216)		(0.005)
Days			
3	1.80	188	0.025
	(0.118)		(0.004)
7	0.439	22.9	<0.01
	(0.091)		
10	0.073	5.87	<0.01
	(0.025)		
14	<0.01	2.37	<0.01
21	<0.01	0.303	<0.01
28	<0.01	0.110	<0.01

[a]Five fish were sampled at each interval.
[b]Pooled bile from five fish.

Table 4. *Mean concentrations of Bayer 73 in plasma and bile (μg/mL) and muscle (μg/g) from rainbow trout exposed to 0.05 mg/L of Bayer 73 for 12 h and transferred to lampricide-free water at 12 ± 1°C for selected withdrawal times (SE in parentheses).*

Withdrawal time[a]	Plasma	Bile[b]	Muscle
Control	<0.01	<0.01	<0.01
Hours			
0	8.11	726	0.146
	(2.29)		(0.027)
4	10.0	313	0.130
	(1.72)		(0.014)
8	8.93	1,000	0.141
	(0.921)		(0.019)
12	7.48	792	0.118
	(0.747)		(0.039)
24	6.93	1,180	0.160
	(1.27)		(0.032)
Days			
3	3.35	117	0.022
	(0.997)		(0.003)
7	0.100	0.95	0.010
	(0.013)		(0.001)
10	0.064	1.45	<0.01
	(0.011)		
14	0.040	1.11	<0.01
	(0.003)		
21	<0.01	0.76	<0.01

[a]Five fish were sampled at each interval.
[b]Pooled bile from five fish.

bile were so much higher, residues did not drop below detectable levels during the selected withdrawal periods in any of the species studied (Tables 3–5). Bile residues had not decreased from initial levels in channel catfish after 14 days of withdrawal (Table 5) and were still detectable (0.11 μg/mL) in coho salmon after 28 days of withdrawal (Table 3).

Residues in bile of the coldwater species exceeded 1,100 μg/mL (Tables 3 and 4), whereas the highest concentrations observed in the warmwater species were only 54 and 126 μg/mL (Table 5).

Table 5. *Mean concentrations of Bayer 73 in pooled samples of bile (μg/mL) and muscle (μg/g) from channel catfish and largemouth bass exposed to 0.05 mg/L of Bayer 73 for 24 h and transferred to lampricide-free water at 19 ± 1°C for selected withdrawal times (SE in parentheses).*

Withdrawal time (days)[a]	Channel catfish		Largemouth bass	
	Bile[b]	Muscle	Bile[b]	Muscle
Control	NS	<0.01	NS	<0.01
0	34.3	0.019 (0.002)	NS	0.035 (0.002)
1	54.0	0.01 (0.001)	NS	0.021 (0.002)
3	49.5	<0.01	126	0.015 (0.000)
7	48.4	<0.01	117	0.015 (0.003)
10	36.8	<0.01	104	0.015 (0.002)
14	35.1	<0.01	50	<0.01

[a]Five fish were sampled at each interval.
[b]Pooled bile from five fish; NS = no sample taken.

Discussion

The maximum observed muscle residue in all species exposed was approximately that of the exposure solution. The decline in muscle residues observed in rainbow trout and channel catfish during uptake exposures (Tables 1 and 2) may have been a result of any one or a combination of the following factors: (1) biotransformation and excretion in the gallbladder bile (Allen et al. 1979; Statham and Lech 1975), (2) decreasing concentration in the exposure bath due to uptake or adsorption by fish, and (3) decomposition (Strufe and Gönnert 1962).

Muscle residues dissipated faster in channel catfish than in the other three species. Brown et al. (1971) suggested that scaleless fish may be capable of transporting chemicals across the integument, in addition to the gill membrane, thereby expediting uptake and elimination. Differences in the ratio of dark muscle to white muscle between open-water and bottom-dwelling species, and associated differences in chemical composition (Love 1970) and vascularization of the two muscle types (Satchell 1971), may also have contributed to the variations observed in rates of uptake and elimination.

From 3 to 14 days of withdrawal were required before residues of Bayer 73 in muscle dropped below 0.01 μg/g. In comparison, Sills and Allen (1975) showed that only 1 day was required for concentrations of TFM to fall below this level.

The continuing increase in bile residues for several hours after coho salmon and rainbow trout were transferred to fresh water, and its maintenance at initial or higher levels in channel catfish for 14 days, were not unexpected. Inasmuch as gallbladder bile is considered to be a major vehicle for collection, storage, and elimination of chemical residues (Lech et al. 1973), various residue pools within the organism may have continued to empty into the bile, even after the fish were transferred to fresh water. Food provided during the withdrawal study probably facilitated the mobilization of residues in the bile. The slow decrease in bile residues suggests that Bayer 73 may have been partly readsorbed and recycled from bile secreted into the digestive tract.

The method of analysis used for this study involves hydrolysis of Bayer 73 to form 2-chloro-4-nitroaniline, which is then analyzed by gas-liquid chromatography. Procedures for analysis of the methyl derivative of the intact molecule in water and sediment were described by Muir and Grift (1980). Fish have been reported to metabolize Bayer 73 by forming a glucuronide conjugate (Statham and Lech 1975; Schultz and Harman 1978; Allen et al. 1979). Although the hydrolysis procedure used in this study would break the glucuronide bond, the method for analysis of residues in muscle tissue measures only free Bayer 73 because the glucuronide conjugate, if present, is lost during the partitioning cleanup steps. Inasmuch as these steps were not used in the analysis of residues in plasma and bile, our values should accurately represent total residues in these fluids.

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Effects of Synergized Rotenone on Nontarget Organisms in Ponds

by

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Abstract

Applications of 2- or 5-μL/L concentrations of synergized rotenone (2.5%) in the Pro-Noxfish formulation to two shallow, 0.05-ha ponds caused a temporary reduction in both total numbers and diversity of benthic invertebrates and a total mortality of caged Asiatic clams (*Corbicula manilensis*) in both ponds, and a partial mortality of a resident population of larval leopard frogs (*Rana pipiens*) in the 5-μL/L treatment. At day 7 after treatment, benthic organisms (no./m^2) were reduced 67% by the 2-μL/L concentration and 96% by the 5-μL/L application. The diversity index ($\bar{d}$) declined sharply in both ponds between days 3 and 7 after treatment, the lowest values being recorded on day 7 and day 37 in the 2- and 5-μL/L treatments, respectively. The equitability index (e) declined from day 3 to day 37 in both ponds. By day 69, however, total numbers of benthic organisms had more than doubled over those originally present in the 2-μL/L treatment (121% increase), had more than tripled in the 5-μL/L treatment (223% increase), and were virtually unchanged in the control pond (2% increase). Pretreatment zooplankton populations were low; no significant deleterious effects from the treatments were observed.

Formulations of rotenone have been used extensively to control fish populations. Concentrations of 0.5 to 1.0 μL/L are adequate in most situations, but rotenone treatments from 0.1 to 5.0 μL/L or more have been used, depending on the purpose of treatment, the fish species present, and conditions at the time of application (Schnick 1974). Chemical and biological conditions in eutrophic waters sometimes require use of the higher concentrations of rotenone (Orn 1962; Spitler 1970). The labels of some rotenone products were recently amended to permit use of concentrations up to 5.0 μL/L. The present study was conducted to evaluate the effects of 2.0- and 5.0-μL/L concentrations of synergized rotenone on benthic invertebrates and zooplankters in ponds. The study was conducted in three experimental ponds at the Warm Springs (Georgia) National Fish Hatchery from 23 August to 1 November 1976.

Methods

The rotenone formulation used was Pro-Noxfish, a synergized emulsifiable concentrate containing 2.5% rotenone, furnished by S. B. Penick & Co., New York, N.Y. This product contains 2.5% sulfoxide, a synergist used to enhance the activity of the rotenone.

The ponds used in this experiment have surface areas of about 0.05 ha, average and maximum depths of about 1.1 and 1.5 m, and clay bottoms. When checked before the start of the tests, each pond supported low populations of zooplankton, moderate populations of phytoplankton and benthic invertebrates, few aquatic plants, and no fish. About 2 weeks before treatment, applications of 20–20–5 fertilizer and hydrated lime were added to each pond to stimulate plankton production, increase pH, and increase total hardness to about 20 mg/L.

On 24 August, ponds I and II were treated with 2- and 5-μL/L applications, respectively, and Pond III served as an untreated control. At the time of treatment, water temperatures were 31–32°C, dissolved oxygen concentrations were 8.5–11.5 mg/L (supersaturated), pH values were 8.6–9.6, and Secchi disk transparencies were 43–80 cm (Table 1). Concentrations of rotenone (active ingredient) applied were 0.05 and 0.125 μL/L. The required volumes of rotenone formulation were diluted in a tub filled with

pond water and then dispensed into the propeller wash of an outboard motor with a boat bailer.

Plankton and benthos samples were taken from each pond before treatment and at 3, 7, 14, 37, and 69 days after treatment. Sampling was terminated after 69 days because populations of most groups of benthic organisms appeared to have recovered from the treatments.

On each sampling date, duplicate samples of benthic organisms were taken from each pond by Ekman dredge (232 cm^2) at 0.3, 0.6, 0.9, 1.2, and 1.5 m in each of the ponds (a total of 10 individually preserved samples from each pond). Samples were washed on a U.S. Standard No. 30 sieve in the field and taken to the laboratory for further washing. The samples were stirred for a few minutes in a saturated aqueous solution of Epsom salts (magnesium sulfate) to facilitate separation of benthos from detritus. Floating organisms and low-density organic materials were then skimmed from the surface. This procedure was repeated at least three times and the remaining mixture of organisms and debris was preserved. Benthic organisms were removed and sorted into major taxa under a binocular microscope. I compared mean numbers of each taxon collected per grab on the several dates with pretreatment numbers, using Student's t test to determine whether observed declines in numbers were significant ($P \leq 0.05$). Both mean diversity ($\bar{d}$) and equitability (e) indices for the benthic invertebrates were calculated for each sampling day in each pond, according to methods outlined by Weber (1973). The major premise on which use of the indices is based is that relatively undisturbed environments support communities having large numbers of species, with no single species present in overwhelming abundance. Declines in $\bar{d}$ and e indices are often associated with adverse influences on benthic communities.

A battery-operated pump with a capacity of 16 L/min was used to collect zooplankton samples at depths of 0.3 and 1.2 m. For each sample collection, 32 L of water were pumped through a Wisconsin plankton net (80-μm mesh) as the boat was moved slowly about the ponds. Samples collected at each depth were concentrated and preserved in 10 mL of a solution consisting of 70% ethyl alcohol, 20% water, and 10% formalin. Numbers of cladocerans, copepods, and rotifers per liter of pond water were determined as suggested by Weber (1973). In addition, 100 adult Asiatic clams, *Corbicula manilensis* (10–14 mm long, 338–985 mg wet wt), were placed in a cage in each pond before treatment and observed for mortalities. Beginning 3 days after treatment, additional cages containing 20 Asiatic clams were placed in the treated ponds on each of 4 successive days. Clams that failed to close their valves or respond to stimuli were considered to be dead.

On the fourth day after treatment, 10 bluegills, *Lepomis machrochirus* (total length, 38–50 mm; weight, 2–4 g), were placed in a cage at a depth of 0.9 m in each pond. All fish were replaced each day until at least eight of the caged fish survived for 24 h.

Temperatures and concentrations of dissolved oxygen were determined at the surface and at 1.5 m on each sampling day. The Secchi disk transparency and the pH at the surface were also recorded in each pond to help ensure that mortalities of invertebrates did not result from adverse water quality.

Results and Discussion

Substantial phytoplankton blooms developed within 3 days after treatment in ponds I and II, and transparency readings were reduced 50% or more (Table 1). Bonn and Holbert (1961) also observed an increase in the number of phytoplankters after the addition of rotenone to lake waters.

Table 1. *Water quality measurements in ponds used to test effects of a synergized formulation of rotenone (2.5% rotenone) on aquatic invertebrates.*

Pond, treatment (μL/L), and sampling day	Water quality measurements				
	Temperature (°C)		Dissolved oxygen (mg/L)		Secchi disk transparency (cm)
	Surface	Bottom	Surface	Bottom	
Pond I, 2.0					
0	31.0	27.0	11.5	10.2	80
3	30.7	27.0	13.2	7.1	40
7	28.0	26.0	>20.0	12.0	27
Pond II, 5.0					
0	31.9	25.1	13.0	2.7	43
3	32.5	26.4	17.2	4.5	15
7	27.8	25.5	>20.0	2.0	18
Pond III, 0.0 (control)					
0	31.0	26.8	8.5	2.0	80
3	30.3	26.9	10.1	6.4	82
7	28.0	27.0	17.2	14.3	80

Table 2. *Estimated total benthic invertebrates (no./m^2) in bottom soil samples before and after treatment of pond I with 2 μL/L of a synergized formulation of rotenone (2.5%).*

Taxa	Days after treatment				
	0	3	7	37	69
Nematoda	0	0	0	17	525
Decapoda (*Procambarus*)	0	0	0	0	9
Ephemeroptera (*Caenis*)	766	159	4	202	474
Odonata[a]	4	13	4	22	0
Coleoptera					
Agabus, Laccophilus	13	69	47	13	26
Dineutus	4	0	0	0	0
Berosus	211	603	56	715	577
Hemiptera					
Notonecta	17	116	0	4	17
Hesperocorixa	30	82	13	34	0
Trichoptera (*Oecetis*)	56	39	0	0	0
Diptera					
Chaoborus	254	164	125	4,585	3,475
Bezzia, Palpomyia	56	112	13	116	422
Glyptotendipes	3,862	2,644	1,507	8,284	6,118
Chrysops	0	0	0	0	4
Total organisms	5,273	4,001	1,769	13,992	11,647

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

They believed that the increase was caused by the reduction in numbers of plankton-feeding fishes, the increased food made available by decaying organic matter, and the additional depth of light penetration. By day 7, dissolved oxygen at the surface in all ponds had increased by more than 50%. The low concentrations of dissolved oxygen (2 mg/L) at the bottom in pond III on day 0 and in II on day 7 caused no mortalities among caged clams and were not considered to have been injurious to the benthic fauna.

All Asiatic clams died within 24 h after treatment in both treated ponds, and there was a substantial mortality of a resident population of larval leopard frogs (*Rana pipiens*) in pond II (5 μL/L). However, among clams introduced into ponds I and II on the third day after treatment, none died within 25 days in pond I (2 μL/L) and only three in II (5 μL/L). No clams or bluegills died in the control pond during the study. Concentrations of rotenone were reduced to essentially nontoxic levels for clams by the fourth day after treatment, but among small bluegills placed in the treated ponds on the fourth day, mortality was 50% in pond I (2 μL/L) and 100% in pond II (5 μL/L). However, degradation proceeded rapidly and both the 2-μL/L and 5-μL/L treatments were nontoxic to bluegills by the sixth day after treatment.

Populations of benthic invertebrates were similar at the outset in that all eight of the orders of organisms were found in all three ponds. The total number of invertebrate taxa initially collected in each pond ranged from 12 to 14, and the estimated total numbers of organisms varied widely among sample days (Tables 2, 3, and 4).

Pronounced changes in total numbers and diversity of benthic organisms occurred in both ponds after treatment, and many dead insect larvae were found in the 3- and 7-day samples. In pond I (2 μL/L), the total number of organisms per unit of area was reduced by 66.5% on day 7. No whirligig beetles (*Dineutus*), backswimmers (*Notonecta*), or caddisflies (*Oecetis*) were found, reducing the total number of orders of insects collected from six to five (Table 2). In contrast, Houf and Campbell (1977) reported that a 2-mg/L application of the Noxfish (5%) formulation of rotenone produced no immediate effects on abundance of benthic organisms in a shallow (0.6 m), mud-bottomed pond that was heavily vegetated. In their study, two factors mentioned by Lindgren (1960) may have contributed to the observed reduction in toxic effects: (1) the oxidation of rotenone in the vicinity of photosynthesizing vegetation; and (2) the sorption of rotenone by gyttja, clay, or gel-mud in the bottom soils. In pond II (5 μL/L), the total numbers of organisms were reduced 96% by day 7; no nematodes, mayflies (*Caenis*), dragonflies (*Libellula, Ophiogomphis, Didymops, Erythemis,* or *Ischnura*), caddisflies, or phantom midges (*Chaoborus*) were found, and the number of orders present dropped from six to two (Table 3). In pond III (control), there was also a pronounced drop in the total number of organisms present on day 7 (Table 4). Apparently, some of this reduction resulted from an emergence of chironomids (*Glyptotendipes*), whose numbers decreased by 48% during the preceding week.

Reductions in abundance of several taxa were observed at various intervals after treatment, but most were not

Table 3. Estimated total benthic invertebrates (no./m²) in bottom soil samples before and after treatment of pond II with 5 μL/L of a synergized formulation of rotenone (2.5%).

	Days after treatment				
Taxa	0	3	7	37	69
Nematoda	13	0	0	17	784
Decapoda (*Procambarus*)	0	0	0	4	0
Ephemeroptera (*Caenis*)	2,174	164	0	125	82
Odonata[a]	168	34	0	138	4
Coleoptera					
Agabus, Laccophilus	30	4	13	0	0
Dineutus	8	0	4	0	0
Berosus	499	133	116	112	52
Hemiptera (*Hesperocorixa*)	0	0	0	4	0
Trichoptera (*Oecetis*)	4	0	0	0	0
Diptera					
Chaoborus	392	56	0	2,458	2,665
Bezzia, Palpomyia	4	4	9	39	215
Glyptotendipes	560	65	17	8,925	8,641
Total organisms	3,852	460	159	11,822	12,443

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

statistically significant. In pond I (2 μL/L), a mortality of mayflies was readily evident, and significant reductions in their numbers were observed on days 3, 7, and 37 (Table 5). Inasmuch as water boatmen (Corixidae) were present in only small numbers on the first four sampling dates, failure to collect them on day 69 was regarded as a sampling anomaly rather than a result of treatment. In pond II (5 μL/L), mayflies and dragonflies were significantly reduced on day 3; on day 7 no mayflies or dragonflies were taken (Table 6). Although both populations increased thereafter, the reduction in their numbers remained significant on all later sampling dates until day 37, when numerous dragonflies were taken (82% of the original number). In the control pond, the numbers of organisms in different taxa fluctuated widely, but no reductions were statistically significant (Table 7).

Recovery from the effects of treatment varied among the different groups of organisms, but was especially rapid

Table 4. *Estimated total benthic invertebrates (no./m²) in bottom soil samples in pond III (control) on days when treated ponds were sampled.*

	Days after treatment				
Taxa	0	3	7	37	69
Nematoda	22	17	99	56	263
Decapoda					
Procambarus	0	0	0	4	0
Palaemonetes	73	73	164	69	103
Ephemeroptera (*Caenis*)	0	0	0	4	0
Odonata[a]	9	13	0	4	4
Coleoptera (*Berosus*)	4	0	4	4	9
Hemiptera					
Notonecta	4	0	0	13	4
Hesperocorixa	0	17	22	0	0
Trichoptera (*Oecetis*)	0	0	0	4	0
Diptera					
Chaoborus	1,085	288	2,316	112	4,994
Bezzia, Palpomyia	99	43	146	336	633
Glyptotendipes	17,110	11,814	8,852	7,552	12,731
Total organisms	18,406	12,265	11,603	8,158	18,741

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

Table 5. *Mean numbers of numerically important groups of benthic invertebrates per grab (standard error of means in parentheses) and percent change from pretreatment numbers after treatment with 2 μL/L of a synergized formulation of rotenone (2.5%) in pond I.*

		Days after treatment							
	Pretreatment	3		7		37		69	
Taxa	Mean number	Mean number	Change (%)	Mean number	Change (%)	Mean number	Change (%)	Mean number	Change (%)
Ephemeroptera (*Caenis*)	17.8	3.7[a]	-79	0.1[a]	-99	4.7[a]	-74	11.0	-38
	(5.2)	(1.3)		(0.1)		(2.1)		(3.9)	
Odonata[b]	0.1	0.3	200	0.1	0	0.5	400	0.0	-100
	(0.1)	(0.2)		(0.1)		(0.5)		(0.0)	
Coleoptera									
Agabus, Laccophilus	0.3	1.6	433	1.1	267	0.3	0	0.6	100
	(0.2)	(0.7)		(0.6)		(0.3)		(0.3)	
Berosus	4.9	17.6	259	1.3	-73	16.6	239	13.4	173
	(3.0)	(8.0)		(0.6)		(5.3)		(5.2)	
Hemiptera									
Notonecta	0.4	2.7	575	0.0	-100	0.1	-75	0.4	0
	(0.2)	(0.7)		(0.0)		(0.1)		(0.2)	
Hesperocorixa	0.7	1.9	171	0.3	-57	0.8	14	0.0[a]	-100
	(0.3)	(1.3)		(0.3)		(0.5)		(0.0)	
Diptera									
Chaoborus	5.9	3.8	-36	2.9	-51	106.5	1,705	80.7	1,268
	(4.7)	(1.4)		(2.2)		(23.3)		(40.0)	
Bezzia, Palpomyia	1.3	2.6	100	0.3	-77	2.7	108	9.8	654
	(0.5)	(0.9)		(0.2)		(1.7)		(5.3)	
Glyptotendipes	89.7	61.4	-32	35.0	-61	192.4	114	142.1	58
	(42.6)	(16.5)		(8.0)		(105.6)		(56.2)	
Total organisms	121.1	95.5	-21	41.1	-66	324.6	168	245.8	103
	(46.0)	(21.7)		(7.2)		(112.3)		(64.9)	

[a]Significant reduction in numbers from pretreatment samples.
[b]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

among dipterans. The density of organisms (no./m^2) in treated ponds on day 37 after treatment exceeded pretreatment populations by 165% in pond I and by 207% in II. In contrast, the numbers in the control pond on day 37 were 56% less than before treatment. One month later (day 69), total numbers of organisms collected in each pond were greater than those before treatment by the following margins: 2-μL/L treatment, 121%; 5-μL/L treatment, 223%; and control, 2%. This finding corresponds with that of Cook and Moore (1969), who observed a rapid and explosive resurgence in insect numbers within 2 to 6 weeks after a late-summer rotenone treatment of a California creek.

Calculation of mean diversity ($\bar{d}$) and equitability (e) indices for the benthic invertebrates showed that treatment caused a substantial decline in $\bar{d}$ values in both ponds I and II by day 7 (Table 8). In pond I (2 μL/L), $\bar{d}$ had increased markedly by day 37, and was slightly higher than the pretreatment value by day 69. In pond II, however, $\bar{d}$ on day 69 remained depressed but the dipteran population (largely *Chaoborus* and *Glyptotendipes*) had increased so much that the total numbers of organisms per square meter greatly exceeded the numbers before treatment. In the control pond, $\bar{d}$ values fluctuated considerably, but were highest on day 69.

The e values in the control pond were consistently lower than those in the treated ponds (Table 8). The e values in both treated ponds were lowest on day 37, and the decline was greatest in the 5-μL/L treatment. The reduction of both $\bar{d}$ and e values indicated that the rotenone treatments disturbed community structure, but a strong trend toward recovery was evident at 69 days after treatment.

Zooplankton populations in all three ponds remained consistently low throughout the study (Table 9). Minor fluctuations in abundance occurred, but the treatments did not produce the pronounced deleterious effects sometimes associated with rotenone applications (Almquist 1959; Kiser et al. 1963). Wollitz (1962) found that a treatment with 0.95 μL/L Pro-Noxfish in one experimental pond greatly reduced the zooplankton population, whereas a 0.70-μL/L treatment in another pond apparently had little effect on most zooplankters. The reason for such differences is unclear. Kiser et al. (1963) suggested that the application of rotenone in spring and early summer, during zooplankton population pulses, appears to have more severe impacts and more lingering effects than applications made in autumn.

Table 6. *Mean numbers of numerically important groups of benthic invertebrates per grab (standard error of means in parentheses) and percent change from pretreatment numbers after treatment with 5 μL/L of a synergized formulation of rotenone (2.5%) in pond II.*

		Days after treatment							
	Pretreatment	3		7		37		69	
Taxa	Mean number	Mean number	Change (%)	Mean number	Change (%)	Mean number	Change (%)	Mean number	Change (%)
Nematoda	0.3	0.0	-100	0.0	-100	0.4	33	18.2	5,967
	(0.2)	(0.0)		(0.0)		(0.2)		(10.0)	
Ephemeroptera (*Caenis*)	50.5	3.8[a]	-92	0.0[a]	-100	2.9[a]	-94	1.9[a]	-96
	(9.6)	(1.5)		(0.0)		(16.6)		(0.6)	
Odonata[b]	3.9	0.8[a]	-79	0.0[a]	-100	3.2	-18	0.1[a]	-97
	(1.3)	(0.4)		(0.0)		(2.2)		(0.1)	
Coleoptera									
Agabus, Laccophilus	0.7	0.1	-86	0.3	-57	0.0	-100	0.0	-100
	(0.4)	(0.1)		(0.2)		(0.0)		(0.0)	
Berosus	11.6	3.1	-73	2.7	-77	2.6	-78	1.2	-90
	(5.9)	(1.9)		(1.2)		(1.1)		(0.4)	
Diptera									
Chaoborus	9.1	1.3	-86	0.0	-100	57.1	527	61.9	580
	(5.7)	(0.7)		(0.0)		(16.8)		(40.8)	
Bezzia, Palpomyia	0.1	0.1	0	0.2	100	0.9	800	5.0	4,900
	(0.1)	(0.1)		(0.2)		(0.3)		(1.3)	
Glyptotendipes	13.0	1.5	-88	0.4	-97	207.3	1,495	200.7	1,444
	(9.5)	(0.6)		(0.2)		(51.6)		(45.3)	
Total organisms	89.2	10.7	-88	4.0	-96	274.5	208	289.0	224
	(18.0)	(3.9)		(1.2)		(63.5)		(37.4)	

[a]Significant reduction in numbers from pretreatment samples.
[b]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

Table 7. *Mean numbers of numerically important groups of benthic invertebrates per grab (standard error of means in parentheses) and percent change in numbers present in pond III (control) on various days after ponds I and II were treated.*

		Days after treatment							
	Pretreatment	3		7		37		69	
Taxa	Mean number	Mean number	Change (%)	Mean number	Change (%)	Mean number	Change (%)	Mean number	Change (%)
Nematoda	0.5	0.4	-20	2.3	0	2.3	160	6.1	1,120
	(0.2)	(0.3)		(0.9)		(0.6)		(1.6)	
Odonata[a]	0.2	0.3	50	0.0	-100	0.1	-50	0.1	-50
	(0.2)	(0.2)		(0.0)		(0.1)		(0.1)	
Coleoptera (*Berosus*)	0.1	0.0	-100	0.1	0	0.1	0	0.2	100
	(0.1)	(0.0)		(0.1)		(0.1)		(0.2)	
Hemiptera (*Notonecta*)	0.1	0.0	-100	0.0	-100	0.3	200	0.1	0
	(0.1)	(0.0)		(0.0)		(0.3)		(0.1)	
Diptera									
Chaoborus	25.2	6.7	-73	54.8	117	2.6	-90	116.0	360
	(20.2)	(3.0)		(27.2)		(0.9)		(54.6)	
Bezzia, Palpomyia	2.3	1.0	-57	3.4	48	7.8	239	14.7	539
	(0.5)	(0.4)		(1.3)		(1.8)		(3.3)	
Glyptotendipes	397.4	274.4	-31	205.7	-48	175.4	-56	295.7	-26
	(99.0)	(82.2)		(81.0)		(41.6)		(68.4)	
Total organisms	425.8	282.8	-34	266.3	-37	187.9	-56	432.9	-38
	(89.8)	(62.7)		(65.8)		(39.9)		(81.3)	

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

Table 8. *Mean indices of diversity ($\bar{d}$) and equitability (e) for benthic invertebrate samples collected at designated times from two ponds treated with a synergized formulation of rotenone and a control pond.*

Pond no.	Concentration of Pro-Noxfish (μL/L)	Index	Pretreatment	Days after treatment 3	7	37	69
I	2.0	$\bar{d}$	1.6229	1.8061	0.8459	1.3656	1.6050
		e	0.4885	0.5038	0.5177	0.4550	0.5511
II	5.0	$\bar{d}$	1.8424	2.1075	0.5548	1.0257	1.2744
		e	0.7771	1.1455	0.8419	0.3484	0.4924
III	0.0	$\bar{d}$	0.4214	0.2625	0.9905	0.4810	1.1799
	(control)	e	0.3039	0.2647	0.4734	0.3286	0.4360

Table 9. *Estimated numbers of zooplankters per liter of water taken at selected intervals from pond III (control) and from ponds I and II, which were treated with 2.0- and 5.0-μL/L applications of rotenone, respectively.*

	Cladocera			Copepoda			Rotifera		
Sampling day	Pond I	Pond II	Pond III	Pond I	Pond II	Pond III	Pond I	Pond II	Pond III
Pretreatment	0.47	0.00	0.00	0.16	0.16	7.03	0.00	0.16	0.00
7	0.00	0.00	0.00	0.00	0.16	2.66	0.63	0.16	0.00
37	0.00	9.84	3.44	0.16	0.16	0.16	0.00	0.00	0.00
69	0.00	1.09	0.16	3.13	2.19	9.84	0.31	0.16	2.03

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(Reports 69 and 70 are in one cover.)

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