

THE TOLERANCE OF EIGHT SPECIES OF WARM-WATER FISHES TO CERTAIN ROTENONE FORMULATIONS¹ *

By F. EUGENE HESTER²

*Agricultural Experiment Station, Alabama Polytechnic Institute
Auburn, Alabama*

ABSTRACT

Laboratory tests were conducted in aquaria to determine the effectiveness of Sulfoxide as a synergist to rotenone for use as a fish poison. In order to compare the synergized product with standard formulations, Pro-Noxfish (2.5 percent rotenone, 2.5 percent Sulfoxide, 5 percent other cube extractives, and an emulsifier) was compared to Noxfish (5.0 percent rotenone, 10 percent other cube extractives, and an emulsifier), and to powdered cube (7.3 percent rotenone).

The relative toxicities of the three formulations were found to vary with water temperature, species of fish, and size of fish tested.

Eight species were tested at approximately 70° F. for 72 hours. The concentrations, expressed in p.p.m. of each formulation, which were required for LD₅₀'s were: carp (Noxfish, 0.081; powdered cube, 0.115; Pro-Noxfish, 0.163); largemouth bass (Pro-Noxfish, 0.081; Noxfish, 0.147; powdered cube, 0.164); fathead minnows (Noxfish, 0.159; Pro-Noxfish, 0.191; powdered cube, 0.200); green sunfish (Noxfish, 0.165; Pro-Noxfish, 0.238; powdered cube, 0.246); goldfish (Noxfish, 0.175; powdered cube, 0.218; Pro-Noxfish, 0.242); bluegills (Noxfish, 0.179; Pro-Noxfish, 0.255; powdered cube, 0.268); golden shiners (Noxfish, 0.470; Pro-Noxfish, 0.555; powdered cube, 0.620); speckled bullheads 1.0 to 1.4 inches (Noxfish, 0.247; powdered cube, 0.346; Pro-Noxfish, 0.410); and speckled bullheads 6 to 8 inches (powdered cube, 0.794; Noxfish, 0.844; Pro-Noxfish, 1.033).

Six species were tested at approximately 40° F. for 72 hours. The concentrations, expressed in p.p.m. of each formulation, which were required for LD₅₀'s were: carp (Noxfish, 0.190; powdered cube, 0.255; Pro-Noxfish, 0.331); fathead minnows (Noxfish, 0.235; Pro-Noxfish, 0.300; powdered cube, 0.417); bluegills (Noxfish, 0.273; Pro-Noxfish, 0.394; powdered cube, 0.417); golden shiners (Noxfish, 0.599; Pro-Noxfish, 0.737; powdered cube, 1.045); speckled bullheads 1.9 to 2.8 inches (Noxfish, 0.602; Pro-Noxfish, 1.003; powdered cube, 1.201); speckled bullheads 3.0 to 3.7 inches (Noxfish, 1.205; Pro-Noxfish, 1.767; powdered cube, 1.971); and goldfish (Noxfish, 1.396; Pro-Noxfish, 2.011; powdered cube, 2.103).

Largemouth bass were killed by smaller concentrations of Pro-Noxfish than either Noxfish or powdered cube, but Noxfish was the most effective of the three formulations against all of the other seven species tested. When compared on the basis of actual rotenone required, all species with possible exception of carp were killed by smaller concentrations of rotenone when Sulfoxide was added, thereby showing a definite synergistic effect of Sulfoxide on rotenone.

Water temperature was found to be an important factor in determining the length of time required for a given concentration to kill the fish. When 40° F. tests were continued beyond the 72-hour test period, the kill of goldfish and fatheads by the end of 21 days was approximately the same as that at the end of 3 days at 70° F., indicating that these toxicants might give the same results at 40° as at 70° F., if given unlimited time, and if the toxicants did not dissipate. When tests were limited to 72 hours, however, more of each rotenone formulation was required to kill fish at 40° than at 70° F.

¹ This is a portion of a dissertation submitted to the graduate faculty of the Alabama Polytechnic Institute, Auburn, Alabama, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June 3, 1959. This project was supported in part by a research grant by S. B. Penick and Company, and was directed by Drs. H. S. Swingle and J. S. Denny.

* Presented at 1958 meeting but not included in Proceedings.

² Resigned September 1, 1959. Present address: Zoology Department, North Carolina State College, Raleigh, North Carolina.

In tests with speckled bullheads and goldfish, large fish were found to be more resistant than small fish to the three rotenone formulations tested.

The addition of 2.0 p.p.m. of potassium permanganate completely detoxified 1.0 p.p.m. of Pro-Noxfish and 1.0 p.p.m. of Noxfish, but only partially detoxified 1.0 p.p.m. of powdered cube (7.3 percent rotenone).

Exposure of 4,000 p.p.m. Noxfish in a glass flask to strong sunlight for 3¼ hours reduced the toxicity to goldfish 66 percent.

INTRODUCTION

Rotenone is important in fisheries management, and its use has become widespread in recent years. Along with this increase in use, improvements have been made in rotenone formulations available for fisheries management. Solvents and emulsifiers have been employed that have enabled fisheries workers to use rotenone in a liquid form, eliminating many of the objectionable features of powdered roots of cube and derris. Recently, chemicals known as synergists or activators have been added to increase the effectiveness of rotenone as a toxicant to fish.

The emulsifiable rotenone formulations used in fisheries management have been standardized to contain 5.0 percent rotenone. Rotenone synergists have been shown to increase the toxicity of rotenone to fish (Price and Calsetta, 1957; Chemical Insecticide Corporation, no date), thus it appears possible that, with the addition of a synergist, formulations containing lesser amounts of rotenone could be used with effects equal to those of 5 percent rotenone. Because the synergists are less expensive than rotenone, reduction of the rotenone content of the formulation should result in a less expensive product.

S. B. Penick and Company has produced a material, Pro-Noxfish, containing 2.5 percent Sulfoxide (1 methyl-2-(3,4-methylenedioxyphenyl)-ethyl-n-octyl sulfoxide), 2.5 percent rotenone, 5 percent other cube extractives, and an emulsifier. In their preliminary tests with goldfish, it appeared to be as effective as Noxfish, their formulation containing 5.0 percent rotenone, 10 percent other cube extractives, and an emulsified (Price and Calsetta, 1957).

To determine more accurately the value of Pro-Noxfish as a fish toxicant, laboratory experiments were conducted in aquaria to compare the toxicities of Noxfish, Pro-Noxfish, and powdered cube (7.3 percent rotenone). The following eight species of fish were tested: carp, *Cyprinus carpio*; largemouth bass, *Micropterus salmoides*; fathead minnows, *Pimephales promelas*; goldfish, *Carassius auratus*; bluegills, *Lepomis macrochirus*; green sunfish, *Lepomis cyanellus*; golden shiners, *Notemigonus crysoleucas*; and speckled bullheads, *Ictalurus nebulosus marmoratus*. The tests were conducted at approximately 70° and 40° F. for each species except bass and green sunfish, which were tested only at 70° F.

GENERAL TESTING PROCEDURE

The fish used in these experiments were seined from ponds, brought into the laboratory, placed in stainless steel troughs provided with flowing water, and treated for control of diseases and parasites. The following treatments, which were used separately, varied only in duration from those recommended by Prather (1957):

1. Potassium permanganate—10 p.p.m. until the fish showed great distress, but never more than 1 hour.
2. Formalin—15 p.p.m. for 6 hours.
3. Acriflavine—1 p.p.m. for 12 hours.

The fish were then graded to obtain test animals of approximately uniform size. The fish were fed Nell's Meat Diet Fish Feed (Evanston Feed Products, Evanston, Illinois) until they were in excellent condition. To minimize contamination of the test water with feces, feeding was discontinued approximately 24 hours before the fish were moved to the aquaria.

The aquaria were washed prior to each experiment and filled with 40 liters of tap water that had been filtered through activated carbon to remove chlorine. For the majority of the tests, 10 fish were used in each aquarium. For all

70° F. tests, an acclimation period of approximately 24 hours was provided, but several days were required to acclimate the fish to 40° F. Because of acclimation difficulties, the 40° F. tests were limited to the colder months of the year.

In experiments using fish 4.5 inches or less in total length, 10 fish were used in each aquarium. Each of four to eight concentrations of each formulation were replicated 4 to 10 times with two replications being conducted during a given test period. Control aquaria were included in all experiments. Several hundred fish, therefore, were required for testing each species with each formulation.

With the exception of one test with 3- to 5-inch goldfish, test with fish larger than 4.5 inches were conducted with fewer than 10 fish per aquarium. With 6- to 8-inch speckled bullheads, only two fish were used per aquarium.

During this study chemical analyses of the water were made an average of 19 times a month by the Alabama Polytechnic Institute's Department of Buildings and Grounds. These analyses were made of the water as it reached the college, but before it had passed through the activated carbon filter in the fisheries building. Records of these analyses revealed a range of pH from 7.8 to 9.4. The total hardness varied from 30 to 50 p.p.m. of calcium carbonate, and the chlorine content varied from 0 to 2.3 p.p.m.

The aquaria were aerated with compressed air, from the time the fish were added until the addition of the test materials, to standardize the dissolved oxygen concentration. The aeration tubes and stones were removed from the aquaria prior to the addition of the toxicants as a precaution against driving off gaseous or volatile components of the solution (Doudoroff, *et al.*, 1951).

The fish in each aquarium were examined several times during the first 24 hours after treatment and at 12-hour intervals thereafter. At each examination, the dead fish were removed and the number recorded. The numbers of fish that were "down" (those fish very severely affected, but not dead) were also recorded. Although the fish were observed during the acclimation period, the actual test period was considered as the 72-hour period after the addition of the toxicant.

TREATMENT OF DATA

When the percent mortality at the end of 72 hours was plotted against concentration, a sigmoid curve was formed, the central portion of which approximated a straight line. At each end of this straight line there was a gradual curve, because the increase in mortality per unit of concentration increase was frequently very low in the regions near 0 or 100 percent mortality. Finney (1952) reported examples of similar type curves. The points between LD_{50} and LD_{90} were used to calculate regression lines presented as graphs in this paper.

RESULTS

In experiments conducted at both 40° and 70° F., mortalities between 10 and 90 percent were used to compute the regression lines. Each line for each formulation was based on 40 to 340 fish of a species.

Control aquaria were present in every test. The number of control fish of each species was approximately half the number used in tests of each formulation. Mortality of large bullheads was 9 percent, but in all other experiments mortality did not exceed 1 percent in control aquaria.

Records of the number of fish "down" in each aquarium at each examination showed that all fish that were "down" did not die. Recovery was made in test solutions without addition of fresh water or any neutralizing material. Because of this, the graphs presented in this paper show only the number of fish dead at the end of the test period. In the 70° F. tests, most of the fish had either died or recovered by the end of 48 hours; therefore, 72 hours appeared to be a desirable test period. At 40° F., however, the fish died very slowly. When preliminary tests with goldfish at 40° F. were extended beyond 72 hours, the fish continued to die slowly for as long as 21 days, at which time the experiments were terminated. Since the deaths in the colder water extended for an indefinite period, 72 hours was chosen as a practical limit.

The results obtained in these experiments are presented graphically. Comparisons of the effectiveness of the three formulations and actual amounts of rotenone required for LD₅₀'s using the three formulations are presented in tabular form.

Fathead Minnows. Six replications were conducted at 70° F. (67° to 74° F.) for 72 hours using fatheads 1.0 to 2.8 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.159 p.p.m.; Pro-Noxfish, 0.191 p.p.m.; and powdered cube, 0.200 p.p.m. (Figures 1 to 3 and Tables I and III.)

Six replications were conducted at 40° F. (37° to 43° F.) for 72 hours using fatheads 1.8 to 2.8 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.235 p.p.m.; Pro-Noxfish, 0.300 p.p.m.; and powdered cube, 0.417 p.p.m. (Figures 4 to 6 and Tables II and IV.)

When experiments were limited to 72 hours, approximately 1.5 to 2.0 times as much of each formulation was required to kill fatheads at 40° as at 70° F. but, when the tests at 40° F. were extended to 21 days, the concentration required for LD₅₀ was approximately the same as at 70° F. in 3 days.

In tests with fatheads at approximately 70° F., for 72 hours, the LD₅₀'s of the emulsifier, solvent, and synergist used in Pro-Noxfish were: Atlox Emulsifier, 16.0 p.p.m.; Sulfoxide (with emulsifier added), 1.57 p.p.m.; and Sovacide (with emulsifier added), 4.4 p.p.m. Thus, it was apparent that Sulfoxide alone was relatively ineffective as a toxicant, but was effective on fatheads as a synergist for the rotenone.

Goldfish. Six replications were conducted at 70° F. (64° to 70° F.) for 72 hours using goldfish 2.0 to 3.5 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.175 p.p.m.; powdered cube, 0.218 p.p.m.; and Pro-Noxfish, 0.242 p.p.m. (Figures 1 to 3 and Tables I and III.)

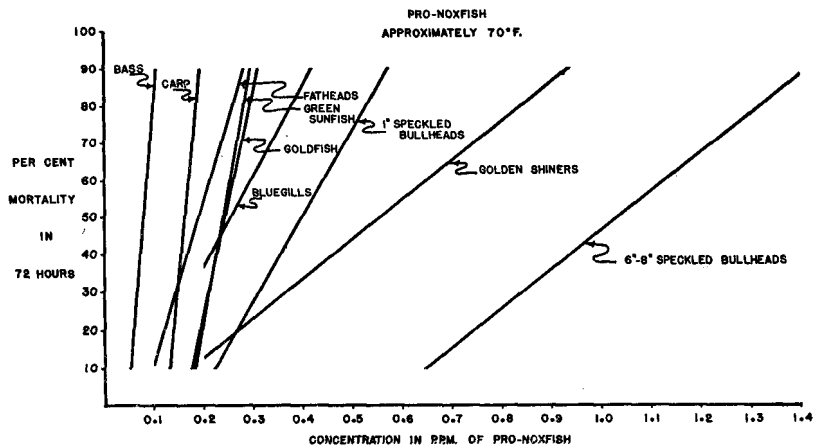


Figure 1. The regression of percent mortality on concentration of Pro-Noxfish at approximately 70° F. for various species of fishes.

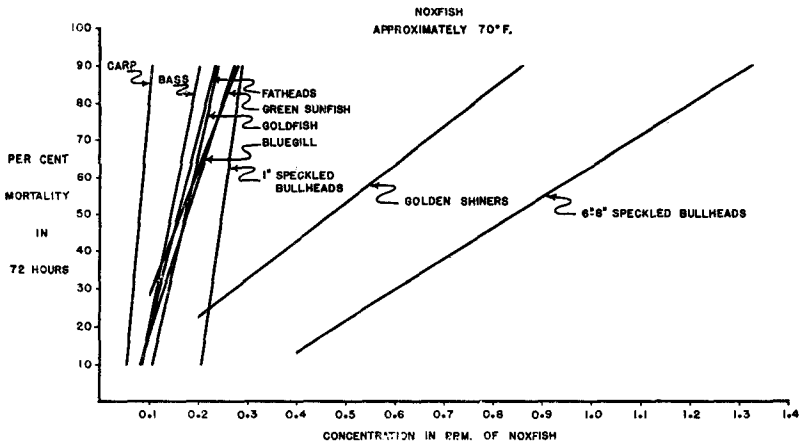


Figure 2. The regression of percent mortality on concentration of Noxfish at approximately 70° F. for various species of fishes.

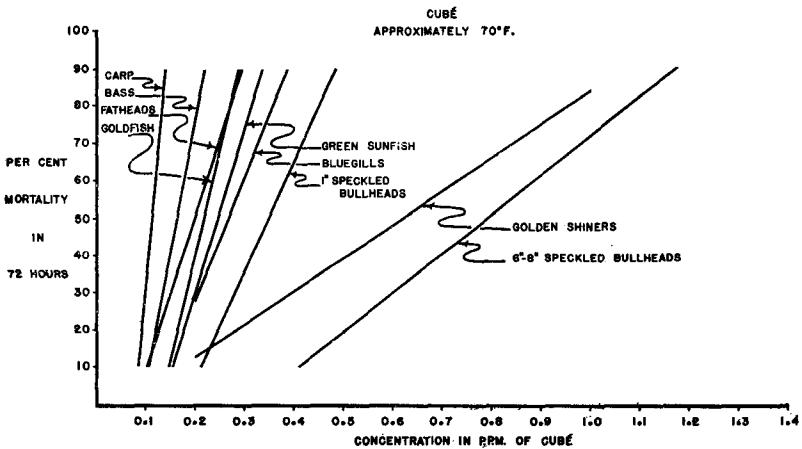


Figure 3. The regression of percent mortality on concentration of powdered cube at approximately 70° F. for various species of fishes.

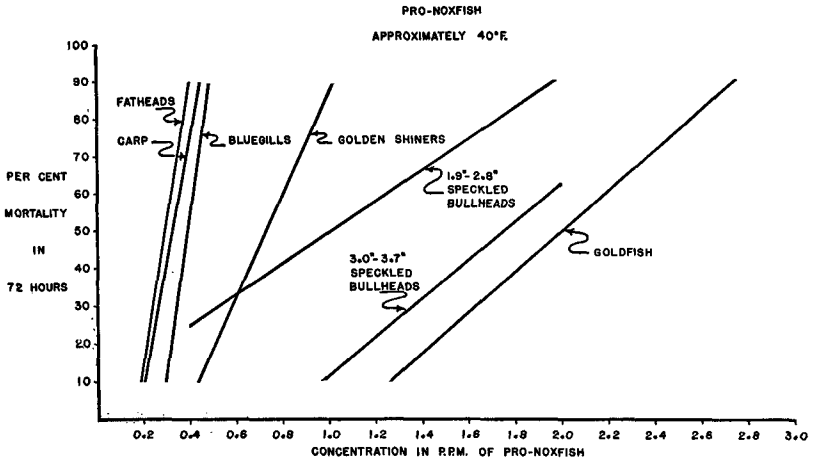


Figure 4. The regression of percent mortality on concentration of Pro-Noxfish at approximately 40° F. for various species of fishes.

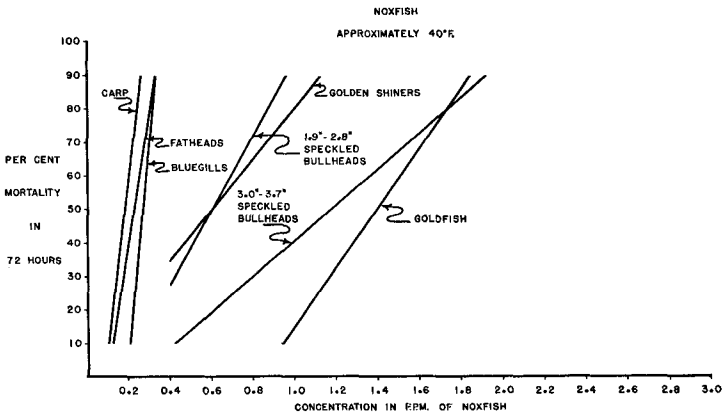


Figure 5. The regression of percent mortality on concentration of Noxfish at approximately 40° F. for various species of fishes.

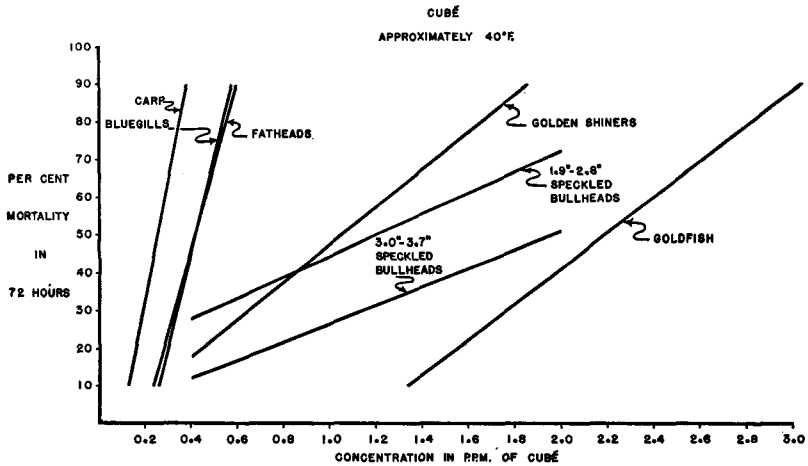


Figure 6. The regression of percent mortality on concentration of powdered cube at approximately 40° F. for various species of fishes.

TABLE I

THE NUMBER OF POUNDS OF NOXFISH OR PRO-NOXFISH EQUIVALENT TO THE KILLING POWER OF 100 POUNDS OF POWDERED CUBE (ADJUSTED TO 5.0 PERCENT ROTENONE), BASED ON VALUES OF LD₅₀ AT APPROXIMATELY 70° F.

Species	Noxfish	Pro-Noxfish
Fatheads	54.4	65.4
Goldfish	55.0	76.0
Carp	48.2	97.1
Bass	61.4	33.8
Bluegills	45.8	65.1
Green Sunfish	46.0	66.2
Golden Shiners	51.9	61.3
Speckled Bullheads 1.0"-1.4"	48.9	81.2
Speckled Bullheads 6"-8"	72.8	89.1

TABLE II

THE NUMBER OF POUNDS OF NOXFISH OR PRO-NOXFISH EQUIVALENT TO THE KILLING POWER OF 100 POUNDS OF POWDERED CUBE (ADJUSTED TO 5.0 PERCENT ROTENONE), BASED ON VALUES OF LD₅₀ AT APPROXIMATELY 40° F.

Species	Noxfish	Pro-Noxfish
Fatheads	38.6	49.3
Goldfish	45.5	65.5
Carp	51.0	88.9
Bluegills	44.8	64.7
Golden Shiners	39.3	48.3
Speckled Bullheads 1.0"-2.8"	34.3	57.2
Speckled Bullheads 3.0"-3.7"	41.9	61.4

TABLE III

THE P.P.M. OF ROTENONE REQUIRED TO KILL 50 PERCENT OF THE FISH USING THE FOLLOWING FORMULATIONS AT APPROXIMATELY 70° F.

<i>Species</i>	<i>Pro-Noxfish</i>	<i>Noxfish</i>	<i>Powdered Cube</i>
Carp	0.004	0.004	0.008
Bass	0.002	0.007	0.012
Fatheads	0.005	0.008	0.015
Green Sunfish	0.006	0.008	0.018
Goldfish	0.006	0.009	0.016
Bluegills	0.006	0.009	0.020
Golden Shiners	0.014	0.024	0.045
Speckled Bullheads 1.0"-1.4"	0.010	0.012	0.025
Speckled Bullheads 6"-8"	0.026	0.042	0.058

TABLE IV

THE P.P.M. OF ROTENONE REQUIRED TO KILL 50 PERCENT OF THE FISH USING THE FOLLOWING FORMULATIONS AT APPROXIMATELY 40° F.

<i>Species</i>	<i>Pro-Noxfish</i>	<i>Noxfish</i>	<i>Powdered Cube</i>
Carp	0.008	0.010	0.019
Fatheads	0.008	0.012	0.030
Goldfish	0.050	0.070	0.154
Bluegills	0.010	0.014	0.030
Golden Shiners	0.018	0.030	0.076
Speckled Bullheads 1.9"-2.8"	0.025	0.030	0.088
Speckled Bullheads 3.0"-3.7"	0.044	0.060	0.144

A differential toxicity according to the size of the goldfish was noticed when one test was conducted at 68° to 76° F. for 72 hours using goldfish 3 to 5 inches in total length. The following mortalities were obtained at 0.4 p.p.m. of each formulation: Noxfish, 50 percent; Pro-Noxfish, 10 percent; and powdered cube, 45 percent. This same concentration was sufficient to kill all of the 2.0 to 3.5-inch goldfish tested.

Goldfish were affected very slowly by the poisons, but in 72-hour tests at 70° F. they were killed by smaller concentrations of the test materials than were several of the other species tested. Although by the end of 72 hours the toxicity lines for goldfish and fatheads were almost identical, the time required to kill goldfish was much greater than that required to kill fatheads (Figure 7). At 20 hours, 0.3 p.p.m. Noxfish had killed 82 percent of the fatheads (average of three tests), while only 15 percent of the goldfish (average of three tests) had been killed.

Six replications were conducted at 40° F. (37° to 42° F.) for 72 hours using goldfish 1.9 to 2.8 inches in total length. The LD₅₀'s for the various formulations were Noxfish 1.396 p.p.m.; Pro-Noxfish, 2.011 p.p.m.; and powdered cube, 2.103 p.p.m. (Figures 4 to 6 and Tables II and IV.)

Because fish in water at 40° F. die more slowly than those at 70° F., the delayed mortality of goldfish becomes of great importance. While only 1.48 times as much Noxfish was required to kill 50 percent of the fatheads in 72 hours at 40° as at 70° F., eight times as much Noxfish was required to kill 50 percent of the goldfish in 72 hours at 40° as at 70° F. When 40° tests were limited to 72 hours, the LD₅₀ for goldfish was 5.9 times that of fatheads. This difference is believed to be primarily a reflection of the delayed mortality of goldfish, since one test that was extended to 21 days required only twice as much Noxfish to kill goldfish at 40° as at 70° F.

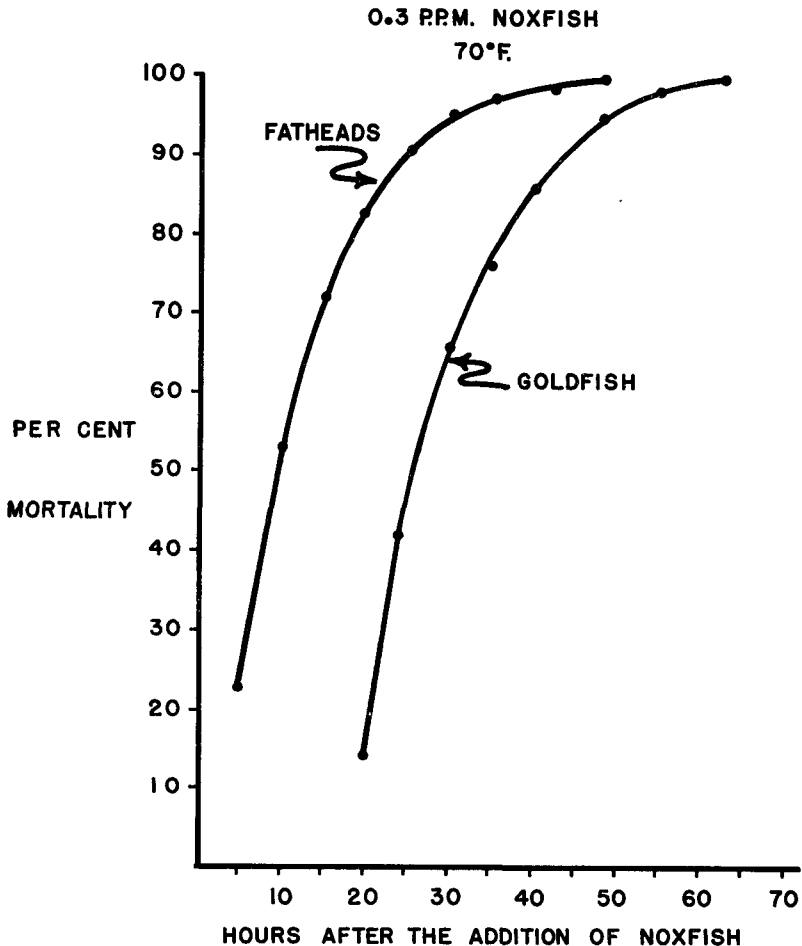


Figure 7. The relative percent mortality in given time interval of fatheads and goldfish when treated with 0.3 p.p.m. Noxfish at approximately 70° F. These curves represent averages of 6 replications of tests on each species.

Carp. Four replications were conducted at 70° F. for 72 hours using carp 1.5 to 2.5 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.081 p.p.m.; powdered cube, 0.115 p.p.m.; and Pro-Noxfish, 0.163 p.p.m. (Figures 1 to 3 and Tables I and III.)

Ten replications were conducted at 40° F. (38° to 43° F.) for 72 hours using carp 2.4 to 3.5 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.190 p.p.m.; powdered cube, 0.255 p.p.m.; and Pro-Noxfish, 0.331 p.p.m. (Figures 4 to 6 and Tables II and IV.) Approximately twice as much of each formulation was required to kill carp in 72 hours at 40° as at 70° F. Also, about twice as much Pro-Noxfish as Noxfish was required to kill carp at both temperatures. Although Sulfoxide had a synergistic action on rotenone for all of the other species tested, there was little if any synergistic action shown for this species.

Largemouth Bass. Ten replications were conducted at 70° F. for 72 hours, using bass 2.0 to 3.5 inches in total length. The LD₅₀'s for the various formu-

lations were Pro-Noxfish, 0.081 p.p.m.; Noxfish, 0.147 p.p.m.; and powdered cube, 0.164 p.p.m. (Figures 1 to 3 and Tables I and III.) When compared with Noxfish at 70° F., only 0.55 as much Pro-Noxfish or 1.12 times as much powdered cube was required to kill 50 percent of the bass. When compared on the basis of actual rotenone required, only 28 percent as much rotenone was required when Sulfoxide was added as in the absence of the synergist. Bass were unaffected by Sulfoxide (with emulsifier added) at a concentration of 0.1 p.p.m., approximately 10 times the LD₅₀ of rotenone. The toxicity of rotenone plus Sulfoxide was much greater than the sum of the toxicities taken independently. Therefore, it is evident from these results that Sulfoxide did have a great synergistic effect and reduced the concentration of rotenone required to kill bass. No tests with small bass were conducted at 40° F., since generally they

Bluegills. Four replications were conducted at 70° F. for 72 hours, using bluegills 1.5 to 2.5 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.179 p.p.m.; Pro-Noxfish, 0.255 p.p.m.; and powdered cube, 0.268 p.p.m. (Figures 1 to 3 and Tables I and III.)

Six replications were conducted at 40° F. (40° to 42° F) for 72 hours using bluegills 1.8 to 3.0 inches in total length. For each of the formulations, 1.53 to 1.56 times as much was required to kill the bluegills at 40° as at 70° F. The LD₅₀'s for the various formulations were Noxfish, 0.273 p.p.m.; Pro-Noxfish, 0.394 p.p.m.; and powdered cube, 0.417 p.p.m. (Figures 4 to 6 and Tables II and IV.)

Green Sunfish. Ten replications were conducted at 70° F. (62° to 74° F.) for 72 hours using green sunfish 1.3 to 3.5 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.165 p.p.m.; Pro-Noxfish, 0.238 p.p.m.; and powdered cube, 0.246 p.p.m. (Figures 1 to 3 and Tables I and III.)

Golden Shiners. Four replications were conducted at 70° F. (70° to 73° F.) for 72 hours using golden shiners 1.5 to 4.5 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.470 p.p.m.; Pro-Noxfish, 0.555 p.p.m.; and powdered cube, 0.620 p.p.m. (Figures 1 to 3 and Tables I and III.)

Four replications were conducted at 40° F. (37° to 50° F.) for 72 hours using golden shiners 1.5 to 2.5 inches in total length. In these tests 1.25 to 1.69 times as much of the three formulations were required to kill 50 percent of the golden shiners at approximately 40° as at 70° F. The Noxfish and Pro-Noxfish toxicity lines crossed, and while Noxfish appeared more effective at 0.4 p.p.m., Pro-Noxfish appeared more effective at 1.0 p.p.m. The LD₅₀'s for the various formulations were Noxfish, 0.599 p.p.m.; Pro-Noxfish, 0.737 p.p.m.; and powdered cube, 1.045 p.p.m. (Figures 4 to 6 and Tables II and IV.)

Speckled Bullheads. Experiments were conducted at approximately 70° F. with speckled bullheads 1.0 to 1.4 inches and 6 to 8 inches in total length. Experiments were also conducted with speckled bullheads at approximately 40° F. using fish 1.9 to 2.8 inches and 3.0 to 3.7 inches in total length.

Four replications were conducted at 70° F. for 72 hours using speckled bullheads 1.0 to 1.4 inches in total length. The LD₅₀'s of the various formulations were Noxfish, 0.247 p.p.m.; powdered cube, 0.346 p.p.m.; and Pro-Noxfish, 0.410 p.p.m. (Figures 1 to 3 and Tables I and III.)

Eight replications were conducted at 70° F. (69° to 78° F.) for 72 hours, using only two speckled bullheads, 6 to 8 inches in total length, in each aquarium. The LD₅₀'s of the various formulations were powdered cube, 0.794 p.p.m.; Noxfish, 0.844 p.p.m.; and Pro-Noxfish, 1.033 p.p.m. (Figures 1 to 3 and Tables I and III.) Powdered cube appeared to be more toxic than Noxfish or Pro-Noxfish, but comparatively few large bullheads could be tested. It seems improbable that Noxfish would be the most toxic formulation to the 1-inch bullheads at 70° F., and to both size groups at 40° F., and yet not be most toxic to large bullheads at 70° F.

Survival of several large bullheads in a concentration of 1.0 p.p.m. of each of the toxicants at 70° F. was considered important. Since this concentration or less has often been used to poison all fish from a body of water, bullheads and golden shiners obviously could have survived such attempts.

Four replications were conducted at 40° F. for 72 hours using bullheads 1.9 to 2.8 inches in total length. The LD₅₀'s for the various formulations were Noxfish, 0.602 p.p.m.; Pro-Noxfish, 1.003 p.p.m.; and powdered cube, 1.201 p.p.m. (Figures 4 to 6 and Tables II and IV.)

The results of four other replications conducted at 40° F. (40° to 43° F.) for 72 hours using bullheads 3.0 to 3.7 inches in total length revealed that the larger fish were more resistant than those 1.9 to 2.8 inches in length. The LD₅₀'s for the various formulations were Noxfish, 1.205 p.p.m.; Pro-Noxfish, 1.767 p.p.m.; and powdered cube, 1.971 p.p.m. (Figures 4 to 6 and Tables II and IV.)

DETOXIFICATION OF PRO-NOXFISH, NOXFISH, AND POWDERED CUBE

Potassium Permanganate. In experiments conducted by Lawrence (1956), the toxicity of 1.0 p.p.m. Noxfish to bass, bluegills, fatheads, and goldfish was effectively counteracted by addition of 2.0 p.p.m. potassium permanganate.

To determine if the toxicity of Pro-Noxfish also could be counteracted by use of potassium permanganate, tests were conducted in aquaria at 68° to 76° F. for 72 hours, using fathead minnows 2.0 to 2.8 inches in total length. Each aquarium was treated with 1.0 p.p.m. Pro-Noxfish, Noxfish, or powdered cube, and after approximately five minutes, some of the aquaria were treated with 2.0 p.p.m. potassium permanganate (KMnO₄). The results were as follows:

<i>Concentration and Material</i>	<i>KMnO₄ Added (p.p.m.)</i>	<i>No. of Fish Tested</i>	<i>Percent Kill</i>
1 p.p.m. Pro-Noxfish	None	30	100
1 p.p.m. Pro-Noxfish	2.0	40	0
1 p.p.m. Noxfish	None	30	100
1 p.p.m. Noxfish	2.0	40	0
1 p.p.m. Powdered Cube (7.3 Percent Rotenone)	None	30	100
1 p.p.m. Powdered Cube (7.3 Percent Rotenone)	2.0	40	100*

* Kill was much delayed, but complete, indicating partial neutralization.

Sunlight. Because field tests indicated that rotenone formulations might be very rapidly detoxified by bright sunlight, a laboratory experiment was conducted to test a stock solution of Noxfish before and after its exposure to bright sunlight.

Eleven aquaria, each containing 10 goldfish (2.5 to 2.9 inches), were prepared in the usual manner for this experiment. After an acclimation period of 20 hours, a stock solution was made by diluting four milliliters of Noxfish to one liter with water in a glass volumetric flask. The stock solution was thoroughly mixed and two milliliters of this solution was applied to each of five of the aquaria (0.2 p.p.m. Noxfish). The stock solution was placed on a concrete walkway outside the fisheries laboratory before noon and allowed to remain in the bright sunlight for 3¼ hours. Two milliliters of this stock solution was placed in each of five additional aquaria. One aquarium remained untreated as a control. The experiment was conducted for 72 hours at 74° to 76° F., and the following results were obtained: before exposure, 66 percent mortality; after exposure, 22 percent mortality.

Exposure of the stock solution to bright sunlight for 3¼ hours reduced the toxicity of Noxfish from 0.20 p.p.m. to 0.13 p.p.m.

DISCUSSION

Since there is a differential toxicity of each rotenone formulation to various species of fish, susceptible species can be killed with a concentration of certain rotenone formulations that will not kill resistant species. Other investigators have found gizzard shad, *Dorosoma cepedianum*, to be highly susceptible to rotenone-containing formulations and have poisoned shad populations in lakes without great harm to other species of fishes (Toole, 1955; Bowers, 1955;

Huish, 1958). The differences in susceptibility appear to be great enough to allow selective poisoning of other species also. The high susceptibility of bass, however, limits the use of these poisons, since bass usually are considered to be a very desirable species. Since this type of selective poisoning removes the susceptible species and leaves the resistant ones, resistant species could not be poisoned without killing other species present.

Even when the undesirable species are less resistant than the desirable ones, there are several factors that prevent direct application of these laboratory results to field poisoning. Application of the toxicant at a uniform concentration throughout a body of water is almost impossible. For that reason, during application some areas would have several times the desired concentration whereas other areas would contain no poison. In addition, sunlight can destroy the toxic effects of these materials, and plankton and suspended soil colloids also may reduce toxicity.

Although powdered cube had a higher rotenone content than Noxfish, it was not found to be as effective as Noxfish against any of the species tested, with possible exception of the 6- to 8-inch bullheads. The stock suspensions of powdered cube were made by mixing it with water and stirring it with a mechanical stirrer. Samples of this stock suspension were applied by pipette to the aquaria. Since the powder was not in a uniform suspension, and samples were taken up by the same method each time, it is possible that some errors resulted from this type of application.

When large fish were tested, the weight of fish per aquarium was increased, and the dosage of the toxicant per kg. of fish was less than when small fish were used. Prevost *et al.* (1948) have shown that, without changing the concentration, the dosage of rotenone per kg. of trout was sublethal when the volume of the mixture was reduced below a certain minimum. Assuming that this also would be true with goldfish and speckled bullheads, the experiments conducted with 10 goldfish (3 to 5 inches) or 2 speckled bullheads (6 to 8 inches) per aquarium may have contained too many fish. In all experiments, the volume of the test solutions was kept constant at 40 liters.

In cold waters all three rotenone formulations generally remained toxic for more than the 72-hour test period. For this reason, with more prolonged exposure, less of each formulation should be required than was indicated by the 72-hour tests.

There may be factors present in the field that increase or decrease the effectiveness of Sulfoxide as a synergist to rotenone. The results presented in this paper will serve as a basis for research to determine and evaluate the factors affecting the toxicity of rotenone to fish under field conditions.

LITERATURE CITED

- Bowers, Charles C. 1955. Selective Poisoning of Gizzard Shad With Rotenone. *Prog. Fish Cult.*, Vol. 17 (3), pp. 134-135.
- Chemical Insecticide Corporation. No Date. *Chem Fish Synergized*. Chemical Insecticide Corporation, 30 Whitman Avenue, Metuchen, New Jersey.
- Doudoroff, P., B. G. Anderson, G. E. Burdick, P. S. Galtsoff, W. B. Hart, R. Patrick, E. R. Strong, E. W. Surber and W. M. Van Horn. 1951. *Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish*. *Sewage and Industrial Wastes*, Vol. 23 (11), pp. 1380-1397.
- Finney, D. J. 1952. *Probit Analysis* (2nd ed.). Cambridge University Press, London. XIV plus 318 pp.
- Huish, Melvin T. 1958. Gizzard Shad Removal in Deer Island Lake, Florida. *Proc. Ann. Conf. S. E. Assoc. Game and Fish Commissioners*. Vol. 11 (1957), pp. 312-318.
- Lawrence, J. M. 1956. Preliminary Results on the Use of Potassium Permanganate to Counteract the Effects of Rotenone on Fish. *Proc. S. E. Assoc. of Game and Fish Commissioners*. (1955) pp. 87-92.

- Prather, E. E. 1957. Experiments on the Commercial Production of Golden Shiners. Proc. Ann. Conf. S. E. Assoc. of Game and Fish Commissioners. Vol. 10 (1956), pp. 150-155.
- Prevost, G., C. Lanouette and F. Grenier. 1948. Effect of Volume on the Determination of DDT or Rotenone Toxicity of Fish. Jour. Wildlife Mgt. Vol. 12 (3), pp. 241-250.
- Price, Robert W. and Douglas R. Calsetta. 1957. Pro-Noxfish A New Synergized Rotenone Formulation for Fish Control. Proc. Ann. Conf. S. E. Assoc. Game and Fish Commissioners. Vol. 10 (1956), pp. 68-75.
- Toole, Marion. 1955. Fish Conservation Highlights of 1954. Sport Fishing Inst. Bulletin. No. 38, p. 49.

LAW ENFORCEMENT SESSION

APPLICATION OF THE LACEY AND BLACK BASS ACTS

By CHARLES H. LAWRENCE

Bureau of Sport Fisheries and Wildlife
Washington, D. C.

I have been asked to discuss the application of the Lacey Act and the Black Bass Act. Before proceeding further I should like to point out that these Acts, although quite different in many respects, have a common beginning and a common purpose. Both were born of national experience, plus national necessity which brought about national action. Their purpose is also the same and that is "to enable the States by their local laws to exercise a power over the preservation of wildlife, which without such legislation they could not exert."

In effect both Acts make use of the potent interstate commerce clause and in a sense extends the States jurisdiction over its property beyond the State's own boundaries.

The Lacey Act is a multi-purpose law and concerns itself with two main objectives: the encouragement of the desirable and the repression of the undesirable. That is to say the Lacey Act was designed to safeguard and improve the status of game birds and other wild birds; to suppress the killing of game as a business, popularly known as market hunting; to make more difficult the slaughtering of various birds, game and nongame, for their plumage; and finally, to regulate the introduction into the country of all exotic species of birds and animals, and rigidly to exclude all such birds and animals known to be dangerous or undesirable.

At the time the Lacey Act was passed (May 25, 1900) it blanketed, so to speak, all of the then outstanding ideas for the halting of the squandering of American wildlife, plus the exclusion of exotic forms of wildlife dangerous to the existing American "balance".

In addition to safeguarding the entire nation from the introduction of dangerous and potentially dangerous birds and animals the Lacey Act was of great benefit to the individual States by putting the market hunter in a very practical hole. The market hunter's name and address had to go on his shipment together with a description of the contents. The transportation companies were now equally liable with the shipper and were joined by the consignee. In other words, the Act provided an opportunity to put a crimp in the illegal transportation of game going, coming, and in between. The game shipper could only start his shipments legally when the local law at the starting point did not forbid its killing. Then he was confronted with a second hazard. As soon as his shipment got within the jurisdiction of its destination it was just as subject to the laws of that jurisdiction as though it had originated therein, and if those laws forbade sale or possession it was liable to confiscation emphasized with a fine.

In 1935 the Act was further amended to meet changing conditions, recognize new methods of transportation, provide police powers not contained in the original Act, increase to one thousand dollars the amount of fine which might be assessed and provide for jail sentences when deemed necessary.