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Carrier Stephens Co.
Mr. Taube
Mr. Washburn

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Controlled tests of the toxicity of Agicide Spray Base derris to fish

by

C. M. Taube and G. M. Washburn

Introduction

Within recent years powdered derris and cube roots (containing rotenone) commonly have been used by biologists for ridding lakes of undesirable fish. For this purpose the Michigan Institute for Fisheries Research has obtained good results by employing concentrations of 0.5 part per million by weight of powdered derris or cube with 5 percent rotenone content. During the war this phase of fisheries management was neglected because of a scarcity of rotenone-bearing substances; but when derris became available again in 1946 work with it was resumed. However, desired aims were not achieved by the Institute in the 1946 season, because the major portion of the new supply of derris, a two-ton lot purchased from the Carrier Stephens Co. of Lansing in August, 1946, proved generally unsatisfactory.

This report concerns: (1) the supply of derris just mentioned; (2) laboratory tests to determine the relative toxicity to fish of another derris preparation (referred to later as Agicide Spray Base).

On August 23, 1946, Nero Lake, Ogemaw County, was treated with a part of the Carrier Stephens supply which, if it had contained 5 percent rotenone, should have produced a complete kill. However, those who engaged in its application reported it was soon evident that expected results were not being attained. Examination of the lake on the day following treatment substantiated the belief that the poison had been only weakly effective because large schools of centrarchid fishes were still swimming about.

Ford Lake in Otsego County was treated with some of the same derris on August 26, 1946. This application gave effective results. It is not known why a discrepancy in results occurred between those of this job and those of the preceding instance and of two instances which follow. It appears possible that a portion of the derris in question may have possessed greater potency than other portions.

A third part of the lot was used to treat East Voelker Pond, Marquette County. August 29 and 30, 1946, were spent on this job. On the first day equipment and material were moved to the pond, which is not conveniently accessible; the derris was applied on the 30th. It was calculated that enough derris was put in the pond to provide a concentration somewhat in excess of 0.5 part per million. The only fish observed to be killed, however, were a few bass in the shallower areas. It was concluded that the treatment had been far from successful. A total of 13 man-days was spent on this job, which was supervised by Dr. A. S. Hazzard, Director of the Institute.

Before the proposed management work on Nero Lake and East Voelker Pond can be continued it will be necessary to treat both with poison again to obtain the complete kill of the fish populations required by the management plans.

A fourth portion of the derris was used on September 5, 1946, to treat the H. O. Wiles minnow pond in Boyne City, Charlevoix County. It was computed that 25 pounds of the powder would be required for a 0.5 p.p.m. concentration; but on the basis of poor results from two previous trials, it was decided to employ a double-strength portion, so 50 pounds were used. However, even by doubling the concentration, the treatment of Wiles' Pond was not completely successful. Although a partial kill of goldfish, suckers, bullheads, fathead minnows, blackchin shiners, and mud minnows occurred, a return visit on September 15 revealed that numerous fish were still alive. George N. Washburn, co-author of this report, supervised the poisoning work.

On October 4, 1946, 25 pounds of derris (rotenone content, 4 percent) were applied to Wiles' Pond. A large number of fish were killed and later observations of the pond showed no fish to be present.

Whereas the derris powder employed in the instances just cited had been purchased with the understanding that its rotenone content was 5 percent, experience indicated the percentage was not that high. Chemical analyses were run on the derris in question and on derris obtained from S. B. Penick & Co., New York, N. Y. The analyses were performed October 30, 1946, in the Pharmacology Department, University of Michigan. The conclusions are quoted below:

> The Penick sample appears to have a slightly lower rotenone content (4.0%) than the label (4.6%) value would indicate. This may be due to loss of rotenone during storage or to the fact that different procedures were used in assaying. (The method used by Penick is not known).
>
> The Carrier-Stevens (sic) sample appears to have a much lower rotenone content (0.83%) than the label value (5.0%) would indicate.
>
> If one assumes that the discrepancy between our results and the label value for the Penick sample are due

to error, and if one further assumes that this error is proportionate in the analysis of the Carrier-Stevens (sic) sample, then the corrected value for the latter would be 0.95% rotenone. This is still far below the label value for this sample.

It would thus appear from our results that the Carrier-Stevens (sic) sample certainly contains not over 1% rotenone.

Frederick H. McKinney
Analyst

Lee Worrell
Supervisor

Obviously the amount of rotenone contained in the Carrier-Stephens product was far too small to give a complete kill with concentrations that usually are employed when derris is used for poisoning fish.

Considerable poisoning work has been scheduled by the Institute for the summer of 1947; it is urgent that a dependable product be obtained for these projects. The Agicide Laboratories, Inc., of Racine, Wisconsin, who supplied the Carrier-Stephens Co. with the derris under discussion, learned of the unsatisfactory results experienced with it and expressed willingness to make an adjustment. A suggestion the firm has made is that it substitute one of their new derris preparations for the lot purchased in 1946. This substance bears the trade name of "Agicide Spray Base". Whereas it is labeled as having a rotenone content of only 1.25 percent, the product was claimed by the Agicide Laboratories to be equivalent to 5 percent rotenone because of a special manufacturing process in which ground walnut shells are used. It was stated that rotenone-bearing roots treated with walnut shell "flour" result in a product permitting "all of the rotenone to be utilized in in insect control". It appeared possible that this claimed increase

in effectiveness, demonstrated in insect control, might also apply when the product is used for killing fish.

The Institute considered testing the effectiveness of "Agicide Spray Base" against fish, and the Agicide Laboratories cooperated by providing two 5-pound bags of the material. During the period February 27 to March 22, 1947, a total of 19 series of experiments was run using "Agicide Spray Base" powder and derris powder, obtained from another source with a rotenone content of 4.0 percent as assayed in the University of Michigan Pharmacology laboratories, and hereafter referred to as the "standard" derris powder. The purpose of these experiments was to test the toxic strength of the Agicide product. The tests were performed in the aquarium room of the Fish Division, University Museums, Ann Arbor.

Materials and Methods

The test fish used in the experiments were creek chubs (Semotilus a. atromaculatus) with a size range of 2.9 to 3.9 inches, average 3.3 inches.

The tests were run in 1-gallon glass jars and 25-quart glass aquaria. The quantities of test suspensions used were 3,000 and 15,000 cubic centimeters for jars and aquaria, respectively. Aeration was accomplished by bubbling compressed air through the derris-water.

In all except one instance comparable tests were run with "Agicide Spray Base" powder and the standard powder already referred to.

For the jars, test concentrations were prepared with stock suspensions of Agicide and "standard" powders by mixing weighed amounts of powder with known quantities of water. Later, definite volumes of these suspensions were mixed with definite volumes of water to make the various test concentrations.

Stock suspensions employed in the experiments included strengths of 15, 16, and 50 parts per million (p.p.m.) of derris. The stock was used at ages of 2, 24, and 48 hours. After all the concentrations for a given test series had been set up, aeration was begun. After 5 minutes of aeration, temperatures were taken and the test fish introduced.

In the aquaria, the derris powders were put directly into the water; stock suspensions were not employed. This procedure was adopted in order more nearly to simulate conditions under which derris is applied to lakes, and also to serve as a check on the jar tests. Fifteen liters of water were put in each aquarium. The test fish were introduced after aeration had begun. Then amounts of derris powder that had been exactly weighed to give desired concentrations were introduced and mixed with the water by hand stirring.

Two types of experiments were run in aquaria, one in which the total amount of powder required for a given concentration was mixed all at once, and one in which the derris for each aquarium had been weighed out in three equal portions which were added at 2-hour intervals.

Water from the Huron River was used for most of the tests, it being thought to approximate the type of water in the majority of Michigan lakes. In addition, some soft water was used, and for one series of tests the stock suspension was prepared with distilled water. Huron River water gave a methyl orange alkalinity reading of 188 p.p.m. of total hardness and a pH of 7.5; the soft water, obtained from the aquarium room supply, gave a methyl orange alkalinity reading of 37 p.p.m. and a pH of 7.5

Experiments in which total mortality did not occur were terminated after from 16 to 24 hours. Experience showed that the minimum time was

more than sufficient to test the toxicity of any concentration. The maximum time required for killing any of the fish that died in these tests was about 9 hours, but in the majority of instances death occurred in considerably less time.

RESULTS

a. Jar Tests.--A preliminary series of five tests in jars was run using "Agicide Spray Base" powder for determining the lethal threshold of this substance. A 15 p.p.m. stock suspension in distilled water was prepared. The 24-hour-old suspension was mixed with soft water when the various concentrations were being prepared. This series placed the lethal threshold at 0.8 p.p.m. for Agicide powder, the lowest concentration in which all fish died. The average time of death was 141 minutes. All fish died in the highest concentration (1.5 p.p.m.) within 60 minutes. A final determination was not obtained for the 1.0 p.p.m. jar because these fish were tested for ability to recover after loss of equilibrium. All fish survived in the two lower concentrations, 0.5 and 0.3 p.p.m.

Table 1. Agicide lethal threshold test, 16 p.p.m. stock suspension mixed with soft water.

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered
1.5	0.01875	2	60	60	60	0
1.0	0.0125	2	...	...	...	2
0.8	0.01	2	40	242	141	0
0.5	0.00625	2	...	...	...	2
0.3	0.00375	2	...	...	...	2

A stock suspension of 16 p.p.m. Agicide derris was prepared with Huron River water; a suspension of the same concentration was made with

the "standard" derris powder. A series of three tests was run on each substance two hours after the stock suspensions had been prepared.

In the Agicide tests, total mortality resulted only in the 0.8 p.p.m. jar. Two of three fish died in the 0.6 p.p.m. concentration, and one of two died in the 0.4 p.p.m.

In tests with the "standard" powder, total mortality occurred in the 0.8, 0.6, and 0.4 p.p.m. concentrations. When comparing the two substances on the basis of the 0.8 p.p.m. concentrations, the "standard" powder was over three times more toxic to fish than the Agicide product.

Table 2.--Agicide relative toxicity test, 16 p.p.m. stock suspension (2 hours old) mixed with Huron River water.

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.8	0.01	4	60	294	118+	0	76°F.
0.6	0.0075	3	77	...	...	1	76°
0.4	0.005	2	99	...	...	1	76°

Table 3.--"Standard" derris powder relative toxicity test, 16 p.p.m. stock suspension (2 hours old) mixed with Huron River water.

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.8	0.032	4	...	34	34	0	76°F.
0.6	0.024	3	...	37	37	0	76°
0.4	0.016	2	...	39	39	0	76°

When the stock suspensions were 24 hours older, two series of tests similar to the preceding were run. Fresh concentrations were

prepared. Only two fish died, both in "standard" derris, indicating that there had been a marked reduction of potency of the stock suspensions after standing 24 additional hours. Here again the greater strength of the "standard" derris was shown by a partial kill and at least temporary loss of equilibrium by the fish in all jars. No fish lost its equilibrium in any of the Agicide jars.

Two-hour and 24-hour tests were run with Agicide and "standard" derris stock suspensions prepared with soft water. These tests are comparable to the four preceding series and were conducted to determine if there might be any difference in retention of potency when stock suspensions are made with waters having different degrees of hardness. No appreciable difference was shown.

Table 4.--Agicide relative toxicity test, 16 p.p.m. stock suspension (soft water & 2 hours old) mixed with Huron River water.

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p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	temperature
0.8	0.01	4	64	267+	122+	0	76°F
0.6	0.0075	3	...	...	...	3	76°
0.4	0.005	2	97	...	...	1	77°

Table 5.--"Standard" derris powder relative toxicity test, 16 p.p.m. stock suspension (soft water & 2 hours old) mixed with Huron River water.

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.8	0.032	4	30	30	30	0	76°F.
0.6	0.024	3	34	39	36	0	76°
0.4	0.016	2	45	67	56	0	76°

Additional tests were performed for determining residual potency in Agicide and "standard" derris by employing considerably higher concentrations. A 50 p.p.m. stock suspension of each substance was prepared. Twenty-four hours later the first series of tests were begun, consisting of 8.0, 4.0, 2.0, and 1.0 p.p.m. concentrations. Total mortality occurred in all the "standard" jars but only in the 8.0 and 4.0 Agicide jars. All test fish survived in the two lower concentrations of the latter. When comparing the results given by the two higher concentrations, the "standard" product was about twice as effective as Agicide derris.

Table 6.--Agicide residual potency test, 50 p.p.m. stock suspension (24 hours old) mixed with Huron River water.

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
8.0	0.1	3	24	31	27	0	77° F.
4.0	0.05	3	41	45	44	0	76°
2.0	0.025	3	...	...	...	3	76°
1.0	0.0125	3	...	...	...	3	76°

Table 7.--"Standard" derris powder residual potency test, 50 p.p.m. stock suspension (24 hours old) mixed with Huron River water.

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
8.0	0.32	3	14	14	14	0	75° F.
4.0	0.16	3	18	23	21	0	75°
2.0	0.08	3	22	27	24	0	75°
1.0	0.04	3	30	↓32	...	1	75°

↓ One specimen of this group was set aside for a revival test.

When the 50 p.p.m. stock suspensions were 48 hours old the tests were repeated. In these no deaths occurred in either Agicide or "standard" derris, although the latter indicated some retention of toxicity as shown by temporary loss of equilibrium of some fish.

b. Aquarium Tests.--Quantities of both Agicide and "standard" derris were weighed out for preparing 0.9, 0.6, and 0.3 p.p.m. concentrations in aquaria. The powder was mixed with the water after the test fish had been introduced. A complete kill in all three aquaria resulted from the use of the "standard" product; complete kills occurred in the 0.9 and 0.6 p.p.m. Agicide aquaria. In all instances the average time required for killing was considerably longer than when fresh suspensions were used in jar tests. Apparently even though the toxic potency of rotenone rapidly decreases beginning some time after contact with water, at the start following mixture, a period of time is required before the point of maximum strength is attained. This seems the most plausible explanation of why it took longer for death to occur in aquaria than in comparable concentrations in jars. In these series the "standard" derris again proved about twice as toxic as Agicide derris.

Table 8.--Agicide relative toxicity test, concentrations prepared directly in aquaria (immediate mixture).

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.9	0.01125	5	192	227	213	0	70° F.
0.6	0.00750	5	205	325	266	0	71°
0.3	0.00375	5	...	...	...	5	72°

Table 9.--"Standard" derris powder relative toxicity test, concentrations prepared directly in aquaria (immediate mixture).

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.9	0.036	5	70	225[1]	110	0	75° F.
0.6	0.024	5	85	201	140	0	76°
0.3	0.012	5	109	279+	216+	0	77°

[1] Four of the five test fish died within a relatively short time of one another (20 minutes between first and fourth deaths). For some unknown reason the fifth lived much longer.

Twenty-four hours later, tests were repeated in the same mixtures prepared the previous day, excepting that no tests were run in the 0.6 and 0.3 p.p.m. Agicide aquaria. Total mortality occurred in the one Agicide aquarium, but the average time of death was more than twice as long as that of the preceding day. No mortality whatever occurred in any of the three "standard" derris aquaria. These series, then, afforded the only instance of the entire experiment wherein the Agicide preparation showed to better advantage than the "standard" derris powder.

Table 10.--Agicide residual potency test, concentrations prepared directly in aquarium (mixture 24 hours old).

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.9	0.01125	5	332	517+	480+	0	72° F.

Table 11.--"Standard" derris powder residual potency test, concentrations prepared directly in aquaria (mixtures 24 hours old).

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES Min.	Max.	Ave.	Survived or recovered	Temperature
0.9	0.036	5	...	...	...	5	75° F.
0.6	0.024	5	...	...	...	5	76°
0.3	0.012	5	...	...	...	5	77°

Other series of tests were run in which fractional portions of both products were added to the aquaria at three different time intervals. Amounts of derris were weighed out so that the total of the three fractions would finally give 0.9, 0.6, and 0.3 p.p.m. concentrations. After the initial fractional quantity had been introduced, the others were added at 2-hour intervals. All fish died in the three aquaria treated with "standard" derris; partial mortality occurred in two aquaria treated with Agicide derris.

Table 12.--Agicide relative toxicity test, concentrations prepared directly in aquaria (fractional mixing).

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES			Survived or recovered	Temperature
			Min.	Max.	Ave.		
0.9	0.01125	5	120	190+	...	1	72° F.
0.6	0.00750	5	190+	305+	...	3	73°

Table 13.--"Standard" derris powder relative toxicity test, concentrations prepared directly in aquaria (fractional mixing).

p.p.m. derris	p.p.m. rotenone	No. of test fish	TIME OF DEATH IN MINUTES			Survived or recovered	Temperature
			Min.	Max.	Ave.		
0.9	0.036	5	145	270	212+	0	74° F.
0.6	0.024	5	210	264	243	0	74°
0.3	0.012	5	250	330	286	0	74°

Summary and Conclusions

As shown by experiment, the lethal threshold (lowest concentration in which all fish died) of Agicide derris was 0.8 p.p.m. in jars and 0.6 p.p.m. in aquaria; "standard" derris produced total mortality in concentrations of 0.3 p.p.m., the lowest concentration

used in these tests. It had been stated that "Agicide Spray Base," labeled as having a rotenone content of 1.25 percent (active toxic ingredient), possessed a potency against insects equivalent to that of other types of derris containing 5 percent rotenone because of a special process employed in its manufacture. However, not nearly so great an increase in potency was shown when the preparation was tested on fish. "Standard" derris, which had been shown by assay in the University of Michigan Pharmacology laboratories to have a rotenone content of 4 percent, definitely was more toxic in four out of five pairs of comparable series of tests. Moreover, even when the two products were compared on the basis of rotenone content, Agicide derris did not measure up to its claimed value as a fish killer.

On the average, the "standard" derris was 2.5 times more toxic than Agicide derris; judging the two products on this basis, the latter appeared more potent than 1.25 percent rotenone would be expected to show. Theoretically, (assuming the rotenone in both powders was equally effective) the "standard" derris powder should have been 3.2 times more effective. This discrepancy, amounting to 22 percent, may be due to various factors:

(1) The "standard" derris may have lost some potency while standing several months after an analysis was made of it; it has been shown that such a loss can occur.

(2) The greater basic strength of "Agicide Spray Base" might be due to some toxic substance other than rotenone; in this instance the substance could occur in the walnut shells. Walnut shells are known to contain an ingredient that is highly toxic to fish.

(3) The special manufacturing process used in preparing "Agicide Spray Base" may have actually increased the effectiveness of its rotenone against fish.

If this difference were due to a foreign toxic substance in the Agicide preparation, it would detract from the value of the product in fisheries work because of the uncertainty of its total effects. Both through experiment and practical application it has been found that rotenone does no appreciable harm to fish food organisms, a consideration of prime importance in this phase of fisheries management. What effect some unknown toxic materials may exert on plankton, insects, and vegetation is not known. The danger of using unproved substances on a large scale is evident.

In conclusion, assuming that the difference in toxicity to fish could be attributed to increased efficiency resulting from the process employed in manufacturing, about two-and-one-half times as much Agicide derris as derris of the "standard" grade still would be required when treating a lake. The larger quantity acquired would increase the cost of application unless the price of the Agicide product were low enough to compensate for the additional poundage required.

It is therefore recommended that the 3520 pounds of Agicide Dust, the unused balance of the order from Carrier Stephens, be returned and that the company be required to replace the ineffective material with an equal weight of powdered derris, cube or timbo root warranted 5 percent rotenone content, as specified in the original requisition and purchase order.

Supplementary Observations

In the tests various observations were made which, while only supplemental to the main purpose of the experiment, nevertheless should be recorded as additional evidence of the effects of rotenone on fish.

Apparently rotenone reacts on fish mainly by impairment of their respiratory functions, which may cause death. In these tests, the first signs of distress were the rising to the surface and gasping of the fish. Equilibrium was gradually lost. In the lower concentrations reactions were never violent, but in concentrations of 1.0 p.p.m. and above (when prepared with relatively fresh stock suspensions) the fish thrashed about vigorously.

After equilibrium had been completely lost, several fish were tested for ability to recover by transferring them to pure water. Complete recovery apparently ensued in specimens from both Agicide and "standard" derris concentrations. Sometimes fish that had lost their equilibrium and appeared doomed to die recovered even while remaining in the derris-water.

Two specimens were affected by paralysis while in Agicide mixtures. The body of one was bent nearly double, causing the chub to swim in a gyrating manner. No curvature developed in the other fish but it could not maintain equilibrium, and also gyrated when swimming. Both specimens were removed from the toxic concentrations after a time and continued to live in fresh water for over 24 hours, although no improvement in condition was shown.

A number of test fish in Agicide and "standard" derris developed hemorrhages. These areas of hemorrhages were irregularly round in

shape, having a maximum diameter of 1 to 2 millimeters; they appeared as collections of blood immediately beneath the epidermis. The hemorrhages occurred on various parts of the body, but appeared more often on the ventral surface and on the lateral areas of the caudal peduncle. Affected fish developed from one to five of such eruptions. Hemorrhaging was highly inconsistent, occurring on some specimens but not on others.

INSTITUTE FOR FISHERIES RESEARCH

C. M. Taube
G. N. Washburn

Approved by: A. S. Hazzard
Typed by: S. E. Benner