E. *Most Desirable and Desirable Trees for Wildlife Management*

Most Desirable Hardwoods	*Desirable Hardwoods*
Turkey Oak	Hickory
Live Oak	Tupelo Gum
Water Oak	Magnolia
Laurel Oak	Beech
Overcup Oak	Maple
Cow Oak	Basswood
Post Oak	Sycamore
Blue Jack Oak	Elm
Black Gum	Ash
Black Cherry	
Other Preferred Tree and Shrub Species	
Iron Wood	Myrtle
Holly	Gooseberry
Dogwood	Sparkleberry
Persimmon	Wild Grapes
Youpon	Virginia Willow

TECHNICAL FISH SESSION

PRO-NOXFISH *

A New Synergized Rotenone Formulation for Fish Control

By ROBERT W. PRICE and DOUGLAS R. CALSETTA

The subject of this report concerns the management of fish populations in our inland lakes and rivers. The condition frequently arises wherein undesirable species will outgrow and overcrowd more desirable types of game fish. In some instances carp will overgrow and seriously damage a waterfowl feeding area by consuming pondweed and other aquatic plants. Also, they so muddy the waters that sunshine cannot penetrate to stimulate growth of new plants.

It has become common practice to treat selected waters for the complete removal of the fish population, then subsequently to restock with game fish. A satisfactory toxicant for the purpose must possess certain attributes among which we can list:

1. High toxicity to fish, low toxicity to mammals.
2. Rapid detoxification without the use of chemicals.
3. Low cost.
4. Readily dispersible.
5. Convenient and safe to use.

Rotenone has been found to be the most desirable toxin for this purpose. It is a chemical which is found in a number of plants belonging to the family *Leguminosae,* especially species of the genera *Derris, Lonchocarpus,* and *Tephrosia.* Lonchocarpus is indigenous to the Amazon Valley and the roots of the plant, commonly known as cubé, are the major source of rotenone for this country.

Although rotenone has been widely used, certain problems arise. Originally applied as a powder (milled cubé root) containing 5% rotenone, operators found it to be irritating to the nose and throat and there were difficulties with dispersion.

Our development of a liquid emulsifiable concentrate was met with enthusiasm. This product could be diluted readily with water at the point of use, and sprayed by hand or by power rigs from boats or even by plane. Reports were received

* Trademark of S. B. Penick & Company. Patents pending.

regarding improved wettability, greater and more uniform penetration, less decrease in effectiveness at colder temperatures, and less labor required for application.

We have now gone one step further by developing a rotenone synergist which lowers the cost by decreasing substantially the amount of rotenone required for a given operation. The finished product which we have named "Pro-Noxfish" is an emulsifiable concentrate containing 2.5% rotenone plus an equal amount of synergist and is used at the same rate as the former concentrate containing 5% rotenone, namely one gallon per 6 acre feet of water (0.5 p.p.m.).

By definition a synergist is a substance which, when combined with another substance, yields an effect which is greater than the sum of the two effects taken independently.

For our experiments to determine a suitable synergist, we set up a laboratory containing a number of five and ten gallon aquaria and a large stock tank. Water was obtained from the tap but was allowed to stand overnight in a storage drum, with aeration to remove traces of chlorine, before being used in any test. Each aquarium was provided with aeration and temperature was maintained at 20-24° C. (68-75° F.) by individual stainless steel cooling coils.

For a test animal we used the common Goldfish, approximately 2" long. The usual procedure was to purchase a week's supply of these fish from a local grower on Friday, hold them over the weekend in the stock tank, and on Monday morning discard all dead, weak, or diseased fish before beginning our tests.

Five fish were used in each 5 gallon aquarium and ten fish in each 10 gallon aquarium. For each test, three emulsifiable formulations were made up:

1. The prospective synergist alone in 20% concentration.
2. The synergist plus rotenone, each in 5% concentration.
3. Rotenone in 5% concentration.

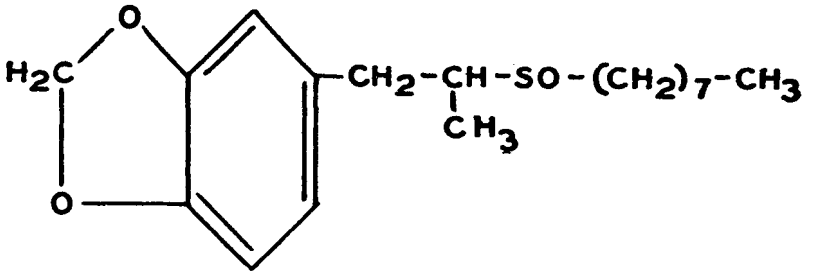
As a source of rotenone, we used soft extract of cubé manufactured by our company and assayed by the official AOAC procedure.

By making suitable dilutions of these formulations and taking proper aliquots, it was possible to introduce variable amounts of the toxicants, expressed as parts per million, into the water.

The procedure used was to make 24 hour counts of the number of dead fish and plot number of deaths against concentration of toxicant in parts per million on log-probability paper. Concentrations were selected by preliminary experiment which resulted in kills from approximately 25% to 75%. The dose resulting in a 50% kill (LD_{50}) was always determined from the graph and used as a measure of the potency for comparison with other toxicants. Experiments repeatedly proved that no mortality was caused by the solvent plus emulsifier alone at ten times the concentrations being used.

The first Table summarizes the data we obtained on sulfoxide.

TABLE I



1-METHYL-2-(3, 4-METHYLENEDIOXYPHENYL)-ETHYL-N-OCTYL SULFOXIDE

Formulation Conc., p.p.m.	*Rotenone Conc., p.p.m.*	*Sulfoxide Conc., p.p.m.*	*No. Fish*	*% Kill*
0.15	0.0075	0	60	86.7
0.10	0.005	0	60	71.7
0.08	0.004	0	60	35.
0.05	0.0025	0	60	18.3
0.075	0.00375	0.00375	60	76.7
0.05	0.0025	0.0025	60	53.4
0.04	0.0020	0.0020	60	31.7
0.025	0.00125	0.00125	60	15.
10.	0	2.0	45	75.6
7.5	0	1.5	45	33.3
6.5	0	1.3	45	20.

When the concentration of toxicant is plotted on log probability paper against percent mortality, it is possible to construct a curve from which the concentration which will cause a 50% mortality can be read. This is illustrated by Graph I for sulfoxide and rotenone.

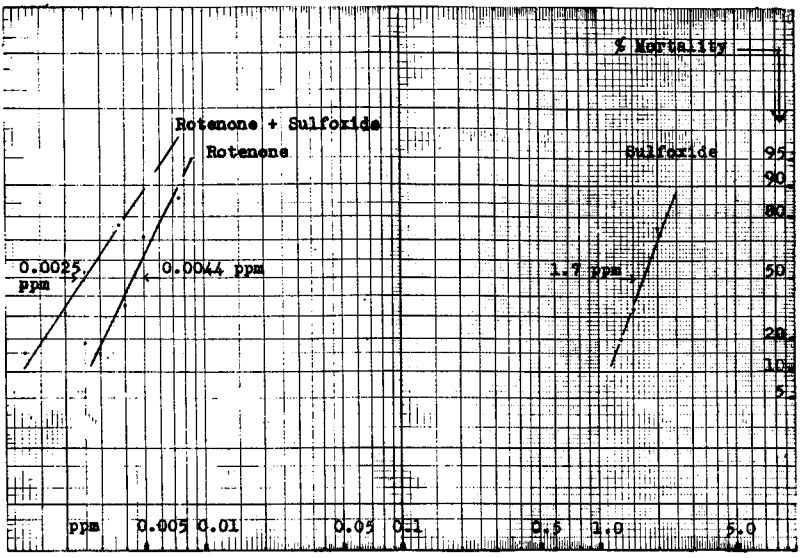
Reading from right to left, the first curve is for sulfoxide alone, which gives an LD_{50} of 1.7 p.p.m. The next curve is for rotenone as it occurs in an extract of cubé root. It has an LD_{50} of 0.0044 p.p.m.

When sulfoxide and rotenone are combined in equal amounts, the LD_{50} becomes 0.0025 p.p.m. as shown by the third curve. This is substantially less than the figure for rotenone alone and is evidence that synergism occurs.

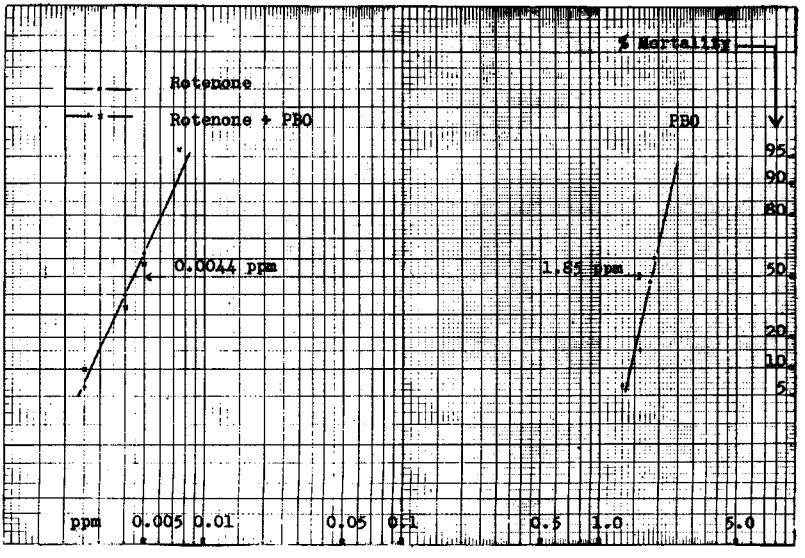
In the case of a substance that is not synergistic to rotenone, there is no lowering in the concentration that produces an LD_{50} and the two curves are superimpossible. This is illustrated by Graph II for piperonyl butoxide.

The curve on the right is for PBO alone, which shows an LD_{50} of 1.85 p.p.m.

Graph I



Graph II



On the left we see that both rotenone and the combination of rotenone plus PBO have the same LD_{50} value of 0.0044 p.p.m., and no synergism occurs.

Table II summarizes our results on a number of compounds tried.

"Sulfoxide"
1-methyl-2(3, 4-methylenedioxyphenyl)-ethyl-n-octyl sulfoxide

"Sulfone"
1-methyl-2(3, 4-methylenedioxyphenyl)-ethyl-n-octyl sulfone

"Thioether"
1-methyl-2(3, 4-methylenedioxyphenyl)-ethyl-n-octyl sulfide

We selected sulfoxide as being the most suitable synergist and next undertook to test our new formulation under field conditions. Arrangements were made to have a number of lakes treated with the new formulation, which we named 'Pro-Noxfish," and then retreated at a later date with our regular 5% rotenone formulation. We reasoned that if the second treatment resulted in no additional kill, the effectiveness of the first formulation would be established.

The following tables document our results:
III. Johnson Sink (Florida)
IV. Chapin Pond (New Hampshire)
V. Jackson (Alabama-Florida)

TABLE II
SUMMARY

	Total Fish	*LD50*			*Synergism*
		Synergist Alone p.p.m.	*Rotenone Alone p.p.m.*	*Synergist+ Rotenone p.p.m. Each*	
Sulfoxide	615	1.7	0.0044	0.0025	+
Sulfone	500	1.9	0.00375	0.0026	+
Thioether	470	1.75	0.0042	0.0042	—
n-Propyl Isome	700	4.2	0.0047	0.0029	+
PBO	310	1.85	0.0044	0.0044	—
MGK-264	575	1.85	0.0056	0,0056	—

Average LD_{50} Rotenone = 0.0045 p.p.m.

TABLE III
JOHNSON SINK—WILLISTON, MARION COUNTY, FLORIDA
Experiment Under Supervision of Edward A. Zagar

Acreage: 11. Acre feet: 88. Deepest point: 12 ft.
Average depth: 8 ft. Type bottom: Hard sand covered with ca. 4" muck.
Plant growth present: None. Condition of water: Clear.

Toxicant	*Pro-Noxfish*			*Noxfish*
Date application	July 30, 1955			August 8, 1955
Temperature surface	85° F.			83° F.
Temperature deepest point	74° F.			74° F.
pH	7.0			7.0
Amount of toxicant applied	15 gal.			15 gal.
Time until first showing of fish	20 min.			.....
Pickup—72 hours	*Wt.*	*No.*	*Species*	None
	1,280	1,000	Chub sucker	
	800	266	Florida gar	"
	620	124	Bowfin	"
	2,700*	1,390		

* In addition there were several hundred pounds of edible fish taken home by interested sportsmen who assisted with this project.

TABLE IV

CHAPIN POND—NEWPORT AND CLAREMONT, NEW HAMPSHIRE

Experiment Under Supervision of Robert B. Knowlton

Acreage: 12. Acre feet: 77. Deepest point: 12 ft.
Average depth: 6.4 ft. Type bottom: Mud and scattered rock and gravel.
Plant growth present: Submerged vegetation (dense). Condition of water: Clear (10.5 ft. Secchi Disk reading).

Toxicant	*Pro-Noxfish*	*Noxfish*
Date application	August 11, 1955	September 8, 1955
Temperature surface	74° F.	67° F.
Temperature deepest point	72° F.	66° F. (at 10 ft.)
pH	6.4	6.6
Amount of toxicant applied	15 gal.	15 gal.
Concentration (app.)	0.58 p.p.m.	0.58 p.p.m.
Time until first showing of fish	15 min.	.....

Pickup—24 hours	*Wt.*	*No.*	*Species*	None
	5.75	34	Brook trout	"
	1.25	1	Rainbow trout	"
	408.0	10,560	Horned pout	"
	415.0	10,595		
	plus "High Kill"	Salamanders		"Few" Salamanders

TABLE V

JACKSON—SOUTH ALABAMA AND NORTH FLORIDA

Experiment Under Supervision of I. B. Byrd, Principal Biologist

Acreage: 372. Average depth: 10 ft.
Plant growth present: A 50-100 ft. wide marginal band of vegetation, mostly maiden cane, pickerel weed, primrose, water lily, and cat-tails.

Toxicant	*Pro-Noxfish*	*Noxfish*
Date application	November 1, 1955	November 10, 1955
Temperature surface	63° F	.....
Amount of toxicant applied	1,500 gal.	1 p.p.m.*

Pickup	*Pounds Per Acre*	
Sucker	15	None
Gar	10	"
Bowfish	5	"
Catfish	5	"
Bass	3	"
Bream	2	"
Eels	3	"
	43	1 Salamander

* In two 4-acre areas at each end of the lake.

The evidence appears to be conclusive that Pro-Noxfish is equal in effectiveness to Noxfish.

Substantial amounts of Pro-Noxfish have already been used in the field. Two of the more interesting applications were (1) Lake Hodges, California and (2) Lake Malheur, Oregon.

Lake Hodges is a 189 acre reservoir, one of eight supplying water for the city of San Diego. The purpose of the treatment was to remove large numbers of carp which caused continuous and excessive turbidity in the lake, to the detriment of the quality of the water. State and County Health Departments gave their permission and the project was controlled and executed by the City of San Diego Water Department. 1,375 gallons of Pro-Noxfish were used.

The project was started January 31, 1956 and the reservoir was reopened for use on February 29, 1956. 109 tons of fish totaling 174,615 mostly carp were collected.

Lake Malheur covers 12,000 acres and is an important link in the Pacific Waterfowl Flyway. The purpose of the project was to eliminate competition between carp and waterfowl for plant foods in this wildlife refuge. The operation was undertaken by the U. S. Fish and Wildlife Service and required about 20 days from mid-October to November 4, 1955. 1,685 gallons of Pro-Noxfish were required and an estimated 1,500,000 carp were recovered.

We next turn our attention to the possible toxicity of Pro-Noxfish to wildlife and domestic animals.

Chronic feeding tests were conducted on groups of 6 to 7 month old lambs over a 4-week period. Pro-Noxfish at levels of 0.5, 5.0 and 50 parts per million was made available in the drinking water, the solutions being made up fresh daily. Table VI summarizes the average daily consumption per animal.

TABLE VI

CHRONIC TOXICITY OF PRO-NOXFISH TO SHEEP

Group	*Number of Lambs*	*Concentration of Pro-Noxfish*	*Volume of Water Consumed Per Lamb Per Day*
1	4	0 (Control)	3,445 c.c.
2	4	0.5 p.p.m.	4,400 c.c.
3	4	5.0 p.p.m.	3,340 c.c.
4	4	50.0 p.p.m.	3,720 c.c.

There were no deaths among any of the groups. The rate of increase in body weight at any dose level was not significantly different from the controls. Blood studies by the School of Veterinary Medicine, University of Pennsylvania were made at the start of the experiment, after 2 weeks and at the end. No adverse effects were noted. Two lambs from the 50 p.p.m. group and one control animal were sacrificed about 1 month after the end of the feeding and autoposies performed by a veterinarian. No pathological changes were observed.

In another experiment conducted by the Wisconsin Alumni Research Foundation, groups of three-week-old White Rock chicks and day old Pekin ducklings were fed ad libitum three concentrations of Pro-Noxfish and one concentration of Noxfish over a period of 4 weeks. The results appear in Table VII.

TABLE VII

DUCKS (15 PER GROUP)

		Average Weight (Grams)					*Avg. Daily Water Consumed (c.c.)*		
Group	*Concentration*	*1 Day*	*1 Wk.*	*2 Wks.*	*3 Wks.*	*4 Wks.*	*2 Wks.*	*3 Wks.*	*4 Wks.*
1	0.5 p.p.m. Pro-Noxfish	53	174	453	796	1,208	363	629	1,040
2	5.0 p.p.m. Pro-Noxfish	51	177	460	799	1,228	374	600	962
3	50.0 p.p.m. Pro-Noxfish	54	168	425	798	1,182	346	582	880
4	25.0 p.p.m. Noxfish	53	173	421	768	1,184	352	573	832
5	Control	53	172	458	819	1,156	378	627	1,077
	CHICKENS (20 PER GROUP)								
1	0.5 p.p.m. Pro-Noxfish	283	416	577	757	928	109	158	177
2	5.0 p.p.m. Pro-Noxfish	273	402	566	745	944	109	147	181
3	50.0 p.p.m. Pro-Noxfish	273	398	559	755	951	106	160	183
4	25.0 p.p.m. Noxfish	279	413	547	766	943	116	155	190
5	Control	271	411	574	746	934	115	147	195

The only mortality was one chick in the control group. No growth differences were apparent and the volume of solution consumed did not differ significantly between groups. At the end of the 4 week period representative subjects were sacrificed and grossly examined. No evidence of toxicity was noted.

In acute experiments the LD_{50} for rabbits was found to be 1.7 ml. of Pro-Noxfish per kilogram of body weight, and for chicks 8 ml. per kilogram of body weight. On this basis it would be necessary for a rabbit to consume 900 gallons of treated water per kilogram of body weight in one drinking to obtain a dose of 1.7 ml. Pro-Noxfish. Similarly a chick would need to consume approximately 4,200 gallons per kilogram.

Pro-Noxfish has been tested by the Laboratory of Industrial Hygiene (New York City) for sensitization on Guinea Pigs according to the Draize procedure. A 0.1% suspension of the sample in physiological saline was injected intravenously 3 times a week for a total of 10 injections. Two weeks after the 10th injection, a retest injection was made. 13 animals were used and there was no evidence of sensitization.

Six months ago we began a chronic toxicity on rats. Approximately 100 weanling rats were divided into four groups. The first group was supplied with water containing 1.0 p.p.m. Pro-Noxfish. The second and third groups were given 10.0 and 100.0 p.p.m. Pro-Noxfish respectively. The last group was given distilled water as a control. We plan to continue this experiment for a year or more, but to date there has appeared no evidence of toxicity even at the highest level and there was no significant difference in weight among these animals

From the evidence that we have obtained it is concluded that Pro-Noxfish is safe for use in water which may be accidently consumed by farm animals or wildlife.

COMPARISON OF TRAWL SAMPLE RESULTS OF MAY, 1953, AND MAY, 1956, ON LAKE GEORGE, ST. JOHNS RIVER, FLORIDA

(Preliminary Report)

By JAMES M. BARKULOO
Florida Game and Fresh Water Fish Commission

INTRODUCTION

A trawl net program was initiated on Lake George, St. Johns River, Florida, in March, 1953 and continued through June, 1953. In May, 1956, the Lake and Stream Survey team of the Florida Game and Fresh Water Fish Commission, duplicated the program as near as possible over a period of one month. A total of 79 trawl hauls were made in May, 1956 for comparison with the same number made in May, 1953. Due to the limited time available for working up the data, only thirty hauls have been completed and compared with 30 hauls made at corresponding stations in 1953. Therefore, this will be a preliminary report; the final report should be completed within the year.

The 1956 trawl net program was a part of an intensive fishery investigation of Lake George to obtain information pertinent to future management of the lake.

HISTORY AND DESCRIPTION OF LAKE GEORGE

Lake George is the largest lake in the St. Johns River drainage having an area of about 73 square miles or 46,600 acres. The major portion of the lake is 9 to 11 feet in depth; the margins are shallow and heavily vegetated with *Vallisneria* sp., *Potamogeton* sp., and hyacinth. For many years it has produced phenomenal sports and commercial fishing. Until 1946, the sale of all species of fish from the lake except largemouth bass was legal. From 1948 to 1950, a fish population survey involving the use of large haul seines was conducted.