INVESTIGATIONS IN FISH CONTROL

39. Effects of Antimycin A on Tissue Respiration of Rainbow Trout and Channel Catfish

40. A Resume on Field Applications of Antimycin A to Control Fish

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

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EFFECTS OF ANTIMYCIN A ON TISSUE RESPIRATION OF RAINBOW TROUT AND CHANNEL CATFISH

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ABSTRACT.--Effects of antimycin A on respiration of liver, kidney, brain, and gill of rainbow trout and channel catfish were measured in vivo and in vitro. In vitro, brain was most resistant to the toxicant, followed by liver and kidney. Brain was more sensitive in vivo than in vitro; liver and kidney were less sensitive in vivo than in vitro. Untreated trout tissues had higher rates of respiration than did catfish tissues. Liver had the greatest oxygen consumption of all tissues.

Early investigations on the mode of action of antimycin A in animals showed it to be an inhibitor of respiration. Ahmad et al. (1949) and Ahmad, Schneider, and Strong (1950) established antimycin A as a potent inhibitor of succinate oxidation in rats. Strong (1958) states that antimycin A is a powerful and selective inhibitor of electron transport in oxidative phosphorylation. Rieske and Zaugg (1962) found almost complete inhibition of Complex III, or reduced coenzyme Q-cytochrome *c* reductase, by antimycin A, and determined that the antimycin-sensitive site is the segment of the respiratory chain which contains cytochrome *b* and c_1. Hepatic aldehyde oxidase is the only enzyme not in the electron transport chain that has thus far been shown to be inhibited by antimycin A (Rajagopalan, Fridovich, and Handler, 1962). Derse and Strong (1963) suggest that its mode of action in fish is also inhibition of respiration. Later, Hiltebran (1965 and 1967) showed that antimycin inhibits succinate and *alpha*-ketoglutarate oxidation in liver mitochondria of bluegills.

Walker, Lennon, and Berger (1964) and Berger[1] found that the toxicity of antimycin A varies among fishes. The families of trouts, perches, and herrings are most susceptible. The pikes, sunfishes, suckers, and cyprinids are of intermediate susceptibility, and the freshwater catfishes, gars, and bowfins least sensitive. Bioassays have shown that channel catfish are approximately 20 times as resistant to antimycin A poisoning as rainbow trout (Walker, Lennon, and Berger, 1964). Although the biochemical mode of action is relatively well defined, the tissue or organ sites of action in fish are not established. Also, whether the sensitivities of trout and catfish tissues to antimycin A are sufficiently different in vitro as well as in vivo to account for species selectivity has not been determined. The present study attempts to determine the tissue response of a sensitive species, the rainbow trout (*Salmo gairdneri*),

[1] Personal communication from Bernard L. Berger, Chemist, Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis.

and a highly resistant species, the channel catfish (Ictalurus punctatus), to antimycin A in vitro and in vivo.

METHODS AND MATERIALS

The rainbow trout used in these investigations ranged from 10.6 to 12.2 inches in length and from 200 to 300 grams in weight and were obtained from the National Fish Hatchery, Manchester, Iowa, and from Peterson Trout Farm, Peterson, Minn. Channel catfish were trapped from the Mississippi River by the National Fish Hatcheries at Guttenberg and Fairport, Iowa, and Genoa, Wis. They ranged from 11 to 13 inches in length and from 138 to 312 grams in weight. Both species were maintained in the laboratory as described by Hunn, Schoettger, and Whealdon (1968). Before use, all fish were acclimated to laboratory conditions in reconstituted water at 12° C. according to the methods of Lennon and Walker (1964).

Antimycin A, 98-percent active ingredient, was obtained from Ayerst Laboratories, New York, N.Y. Stock solutions for in vitro studies were prepared by dissolving antimycin A in sufficient 95-percent ethanol and diluting to volume with 50-percent ethanol. The stocks for in vivo tests were prepared by dissolving antimycin A in appropriate amounts of 100-percent acetone so that aliquots added to the test media did not exceed 1 milliliter per liter.

In vitro studies

The fish were killed by severing the spinal cord behind the head. Livers, kidneys, and brains were removed, and a small sample excised for determination of dry weight. The remainder was weighed, mixed with reaction medium, and hemogenized in a teflon homogenizer. The final homogenate consisted of one part tissue (wet weight) to seven parts reaction medium. The reaction medium of Anthony and Munro (1964) was modified to contain 0.25M sucrose, 0.04M magnesium chloride, 0.02M potassium chloride, and 0.004M EDTA (tetrasodium salt). This medium gave the most consistent and highest metabolic rates, for the tissues involved, of all media tested.

Two ml of each homogenate were placed in a standard single-arm reaction vessel with 0.2 ml of 10-percent potassium hydroxide absorbed on filter paper in the center well. One to 10 microliters of antimycin stock were added to the homogenate already in the vessels, depending on the desired concentration. Earlier, we determined that these amounts of ethanol had no effect on respiration or readings. The vessels were then placed on the manometers of a Warburg apparatus and equilibrated for 15 minutes at 25° C. Air served as the gaseous medium. No more than 10 minutes elapsed between tissue dissection and the start of equilibration.

After equilibration, the manometers were closed, and measurements were made according to the methods of Umbreit, Burris, and Stauffer (1964). Respiration rates were determined as milliliters of oxygen uptake for 1 hour per milligram dry weight of tissue ($QO_2 = O_2$ uptake per hour per mg dry weight). Two reaction vessels, one with untreated homogenate and another with reaction medium, served as controls during manometric determinations. Each experiment was replicated four or nine times, and the mean and standard deviation computed by standard methods. Analysis of variance and the method of least significant differences were used to determine whether antimycin A had caused a significant reduction (0.05 level of significance) in respiration of treated tissues compared with control values.

In vivo studies

Polyethylene tanks containing 45 liters of reconstituted water at 12° C. were used for in vivo exposures of trout and catfish to antimycin A. One fish was placed in each tank and exposed to the toxicant for 4 hours. The concentrations of antimycin A tested ranged between 3 and 80 parts per billion. Controls were exposed to corresponding concentrations of acetone solvent. The behavioral responses of the fish during exposure were recorded, and after 4 hours the fish were killed and the tissues processed as in the in vitro experiments. The respiration of gill tissue was measured only in vivo. Two gill bars with filaments were removed from exposed fish, weighed, and placed in 2 ml of reaction medium. Gill respiration was measured as oxygen uptake per hour per mg wet weight of gill.

RESULTS

Among untreated control fish, the tissues of rainbow trout generally had higher rates of respiration than those of channel catfish at 25° C. (tables 1 and 2). This might be expected since Beamish (1964) has shown that at 20° C. the respiration of brook trout is approximately double that of brown bullheads. Oxygen consumption was greatest in liver tissue, followed by brain and kidney. The low rates of oxygen uptake by gill tissue (table 3) are not comparable to those of other tissues because they are based on wet weight.

The in vitro effect of antimycin A on the rate of oxygen uptake by tissues of rainbow trout and channel catfish appears more consistent between species than between tissues of the same species. Concentrations of 5 ppb or more of antimycin A cause a statistically significant inhibition of kidney respiration in both trout and catfish, while 10 ppb and 20 ppb interfere significantly with liver respiration (table 1). The brains of both species were approximately 4 to 8 times as resistant to the toxicant as liver. A concentration of 80 ppb lowered oxygen uptake of trout brain by 54 percent and of catfish brain by 33 percent. Although the major differences are between tissues, the degree of inhibition in catfish liver and brain is somewhat less than in trout. This suggests that tissues of the former species may be more resistant to antimycin A.

Rainbow trout and channel catfish were exposed to antimycin A in water to determine whether the concentrations of toxicant inhibiting tissue respiration in vitro also inhibited the respiratory rates of these tissues in vivo. The fish were exposed to different concentrations of the toxicant for 4 hours at 12° C. During this exposure, no observable effects were detected at concentrations of 3 ppb on trout or 10 ppb on catfish. However, at 5 ppb the trout began surfacing after 1 to 2 hours, then settled to the bottom in a state of sedation. A similar reaction occurred in catfish within 1 hour at concentrations of 20 and 40 ppb. At 4 hours the trout had lost equilibrium and were lying on their sides, but opercular movements continued. Trout exposed to 10 ppb and catfish to 80 ppb showed symptoms of antimycin poisioning within 30 minutes and were moribund at 4 hours.

The metabolic rates of liver, kidney, brain, and gill of the above fish were measured at 25° C. In three instances, liver and kidney of trout and brain of catfish, the inhibition of respiration in vivo was similar to that in vitro (tables 1 and 2). The most striking differences between in vitro and in vivo effects of antimycin A were in the brain of trout and the liver and kidney of catfish. The concentrations causing significant inhibition in trout brain were 80 ppb in vitro compared with 10 ppb in vivo, whereas the in vitro concentrations for catfish liver and kidney were fourfold those in vivo. Gill and kidney tissue have approximately the same sensitivity to antimycin A in vivo, but the gills of catfish are between 4 and 8 times as resistant as those of trout (table 3).

DISCUSSION

The sensitivities in vitro of rainbow trout and channel catfish tissues to antimycin A are not sufficiently dissimilar to account for differences in its toxic effect on these fishes in the order of magnitude reported by Walker, Lennon, and Berger (1964), but our in vivo tests confirm the relatively high resistance of channel catfish. Thus, antimycin A is either poorly absorbed or is more readily deactivated or metabolized by catfish.

Differential rates of absorption may be linked to variations in gill structure. Steen and Berg (1966) noted significant differences between the gill structures of active fishes such as brown trout (*Salmo trutta*) and European perch (*Perca fluviatilis*), and relatively inactive species like the brown bullhead (*Ameriurus nebulosus*) and eel (*Anquilla*

Table 1.--In vitro effect of antimycin A on the respiration of three tissues of rainbow trout and channel catfish at 25°C.

Species, tissue, and concentration of antimycin	Number of fish	Oxygen uptake in µl. of O_2 per mg dry weight per hour				
		Mean rate	Standard deviation (±)	Change from control	Percent change	Significant 0.05 level
Rainbow trout:						
Liver:						
Control	10	3.26	0.28	--	--	--
3 ppb	10	3.24	0.42	-0.02	0	No
5 ppb	10	3.26	0.24	0	0	No
10 ppb	10	1.32	0.10	-1.94	59	Yes
20 ppb	10	1.04	0.24	-2.22	68	Yes
Kidney:						
Control	10	2.12	0.25	--	--	--
3 ppb	10	2.18	0.15	+0.06	3	No
5 ppb	10	1.54	0.11	-0.58	27	Yes
10 ppb	10	0.68	0.12	-1.44	68	Yes
20 ppb	10	0.45	0.10	-1.67	79	Yes
Brain:						
Control	5	2.51	0.21	--	--	--
5 ppb	5	2.51	0.07	0	0	No
10 ppb	5	2.40	0.26	-0.11	4	No
20 ppb	5	2.41	0.11	-0.10	4	No
40 ppb	5	2.38	0.18	-0.13	5	No
80 ppb	5	1.16	0.34	-1.35	54	Yes
Channel catfish:						
Liver:						
Control	5	1.81	0.05	--	--	--
5 ppb	5	1.66	0.15	-0.15	8	No
10 ppb	5	1.83	0.24	+0.02	1	No
20 ppb	5	0.97	0.15	-0.84	46	Yes
Kidney:						
Control	5	1.44	0.32	--	--	--
5 ppb	5	1.04	0.15	-0.40	28	Yes
10 ppb	5	0.43	0.09	-1.01	70	Yes
20 ppb	5	0.27	0.09	-1.17	81	Yes
Brain:						
Control	5	1.51	0.48	--	--	--
20 ppb	5	1.65	0.81	+0.14	8	No
40 ppb	5	1.47	0.14	-0.04	3	No
80 ppb	5	1.01	0.21	-0.50	33	Yes

vulgaris). They found that the lamellae on gills of inactive fish were fewer in number and thicker than those of the active fish. They calculated the diffusion distance, or distance between blood and water, in bullheads as approximately three times that in trout. Further, the investigations of Steen and Kruysse (1964) indicated that a circulatory shunt system is present in the gill lamellae of marine and freshwater teleosts. Of the freshwater forms they studied, including rainbow trout and the catfish (Silurus glanis), the system was most highly developed in Silurus. Adrenaline caused blood to circulate through the lamellae, but in the presence of acetylcholine blood passed through a central compartment directly between afferent and efferent arteries. Therefore, the resistance of catfish to antimycin A may be related to a well-developed shunt system which is enhanced by the antimetabolic action of antimycin A, and to a thicker gill epithelium.

The in vivo and in vitro effects of antimycin A are more nearly alike in rainbow trout than in channel catfish. However, the greater sensitivity of trout brain in vivo may reflect secondary physiological effects of antimycin which are related to the biochemical actions described by Rieske (1967). Hunn[2] found that 0.2 mg per kg body weight of antimycin A injected intraperitoneally into carp reduced urine flows, but the urine contained elevated amounts of sodium, potassium, calcium, and

[2] Personal communication from Dr. Joseph B. Hunn, Fishery Biologist, Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis.

TABLE 2.--In vivo effect of antimycin A on the respiration of three tissues of rainbow trout and channel catfish at 25°C.

[Oxygen consumption was measured at 25°C., tissues collected from fish which were exposed to antimycin A at 12°C.]

Species, tissue, and concentration of antimycin	Number of fish	Oxygen uptake in µl. of O_2 per mg dry weight per hour				
		Mean rate	Standard deviation (±)	Change from control	Percent change	Significant 0.05 level
Rainbow trout:						
Liver:						
Control	10	4.12	0.39	--	--	--
3 ppb	10	3.81	0.28	-0.31	7	No
5 ppb	10	3.96	0.26	-0.16	4	No
10 ppb	10	0.76	0.32	-3.36	82	Yes
Kidney:						
Control	10	2.37	0.16	--	--	--
3 ppb	10	2.16	0.17	-0.21	9	No
5 ppb	10	2.02	0.47	-0.35	15	Yes
10 ppb	10	0.84	0.39	-1.53	64	Yes
Brain:						
Control	5	2.36	0.18	--	--	--
3 ppb	5	2.42	0.06	+0.06	2	No
5 ppb	5	2.42	0.25	+0.06	2	No
10 ppb	5	0.65	0.25	-1.71	72	Yes
Channel catfish:						
Liver:						
Control	5	1.62	0.32	--	--	--
10 ppb	5	1.53	0.44	-0.09	6	No
20 ppb	5	1.74	0.27	-0.12	7	No
40 ppb	5	1.65	0.12	-0.03	2	No
80 ppb	5	1.30	0.28	-0.32	20	Yes
Kidney:						
Control	5	1.11	0.61	--	--	--
10 ppb	5	1.23	0.17	+0.12	10	No
20 ppb	5	0.76	0.16	-0.35	31	Yes
40 ppb	5	0.75	0.20	-0.36	32	Yes
80 ppb	5	0.56	0.19	-0.55	49	Yes
Brain:						
Control	5	1.57	0.14	--	--	--
10 ppb	5	1.50	0.15	-0.07	4	No
20 ppb	5	1.57	0.27	0	0	No
40 ppb	5	1.49	0.17	-0.08	5	No
80 ppb	5	0.78	0.12	-0.79	50	Yes

magnesium. Schoettger and Svendsen (1968) indicate that antimycin A disrupts the acid-base balance in rainbow trout and channel catfish. A concentration of 2 ppb reduced the total carbon dioxide content of trout blood from 26 to 9 volumes percent during a 6-hour exposure. The blood pH in these fish dropped from 7.4 to 6.9, and lactate increased from an average of 43 to 184 mg percent. Similar changes occurred in channel catfish exposed to 80 ppb for 3 hours. In later unpublished studies, we observed that the effects of antimycin A on acid-base balance in catfish were less acute until just before death. This may account for the apparently greater anesthetic effect of antimycin A on trout.

The brain tissue of mammals also appears resistant to antimycin A. According to a review by Rieske (1967), the toxicant is a potent in vitro inhibitor of succinate oxidation in rat heart, kidney, brain, muscle, spleen, thymus, lung, and tumor. However, inhibition is proportional to enzyme activity in the tissue. Tissues with low succinate oxidase titer, such as spleen, lung, and thymus, were strongly inhibited in vivo. Those with high titers, such as heart, brain, and muscle, were barely affected. Liver was an exception, with a high titer and strong inhibition. The in vivo resistance of rat brain to antimycin A was explained on the basis of the blood-brain barrier. Thus, the contrasting resistances of trout and catfish brain in vivo may reflect the influence of hematological changes induced in trout by relatively low concentrations of antimycin A.

TABLE 3.--In vivo effect of antimycin A on the respiration of gill tissue of rainbow trout and channel catfish at 25°C.

[Oxygen consumption was measured at 25°C., tissues collected from fish which were exposed to antimycin A at 12°C.]

Species and concentration of antimycin	Number of fish	Oxygen uptake in µl. of O_2 per mg wet weight per hour				
		Mean rate	Standard deviation (±)	Change from control	Percent change	Significant 0.05 level
Rainbow tout:						
Control	10	0.125	0.019	--	--	--
3 ppb	10	0.126	0.009	+0.001	1	No
5 ppb	10	0.094	0.034	-0.031	25	Yes
10 ppb	10	0.015	0.008	-0.110	88	Yes
Channel catfish:						
Control	5	0.099	0.027	--	--	--
10 ppb	5	0.094	0.022	-0.005	5	No
20 ppb	5	0.087	0.022	-0.022	12	Yes
40 ppb	5	0.062	0.022	-0.037	37	Yes
80 ppb	5	0.036	0.011	-0.063	64	Yes

Catfishes are apparently able to exclude, detoxify, or otherwise accommodate antimycin. Resistance by exclusion was discussed earlier. However, Ritter and Strong (1966) indicated that the more resistant carp (Cyprinus carpio) survived longer and took up more tritium-labelled antimycin A internally than the more susceptible rainbow trout. If a portion of the radioactive residue in carp was intact antimycin, it must therefore be absorbed, but possibly at different rates since the carp survived much longer. Conceivably, limited uptake coupled with detoxication contributes to catfish resistance.

Rieske et al. (1967) demonstrated that Complex III of the mitochondrial respiratory chain is irreversibly inhibited in vitro by antimycin A. Yet, in a review of antimycin A, Rieske (1967) records other investigations which show that the succinate oxidase systems of rat liver, lung, and spleen inhibited in vivo by sublethal doses recover completely within 2 to 4 hours. Serum albumin was found to bind antimycin A and was implicated as the factor responsible for reactivation of succinate oxidase. The in vivo reactivation of respiration may result from transfer of antimycin A from the inhibited site to serum albumin and its subsequent excretion or inactivation after disassociation from albumin. Liver was the only tissue capable of chemically inactivating antimycin A in vitro. He explains this apparent discrepancy in the reversibility of antimycin A inhibition on the basis of a possible low requirement of active Complex III to support a maximal rate of succinate oxidation. So a relatively small reactivation of this complex may be sufficient to support full succinate oxidase activity.

To date, there has been no indication of a bypass of antimycin A-inhibited components in vertebrates which could contribute to the resistance of catfishes. Takemori and King (1964) report the reversal of antimycin-inhibited succinate cytochrome c reductase from rat heart muscle by coenzyme Q_2. Antimycin A and coenzyme Q_2 appear competitive with regard to inhibition and reactivation respectively (Rieske, 1967), and this precludes a mechanism of reactivation involving an electronic bypass. However, Cheah (1967) reported a branched electron transport system in the cestode Moniezia expansa which might serve as a bypass when the normal antimycin-sensitive site was inhibited. Whether such a bypass less sensitive site or alternate pathway exists in catfishes has yet to be elucidated. Comparative investigations of biochemical and physiological mechanisms and their relation to rates of uptake and detoxication are essential to an understanding of resistance in fishes and the concurrent development of fish-control agents.

SUMMARY

The effects of antimycin A poisoning on oxygen consumption in tissues of a sensitive

species, rainbow trout, and a resistant species, channel catfish, were measured by means of a Warburg respirometer. Oxygen consumption was measured from gill exposed to antimycin A in vivo, and from liver, kidney, and brain exposed both in vivo and in vitro, at various concentrations of the toxicant.

Untreated trout tissues generally had higher rates of respiration than those of catfish at 25° C. Oxygen consumption was greatest in liver, followed by brain and kidney. In vitro effects of antimycin A were more consistent between species than tissues. Brain is four to eight times as resistant to the toxicant as liver in both species.

Trout exposed in vivo to 10 ppb and catfish to 80 ppb of antimycin A showed signs of poisoning within 30 minutes and were moribund after 4 hours at 12° C. Inhibition of liver and kidney of trout and brain of catfish in vivo was similar to that in vitro. However, trout brain was eight times as sensitive in vivo, whereas catfish liver and kidney were about one-fourth as sensitive. Catfish gill tissue was over four times as resistant than trout.

REFERENCES

Ahmad, K., F. M. Bumpus, B. R. Dunshee, and F. M. Strong.
1949. Antimycin antibiotics. Federation Proceedings, vol. 8, p. 178-179.

Ahmad, K., H. G. Schneider, and F. M. Strong.
1950. Studies on the biological action of antimycin. Archives of Biochemistry, vol. 28, p. 281-284.

Anthony, Adam, and Donald W. Munro.
1964. Cellular energetics in liver and kidney homogenates of carp (Cyprinus carpio). Proceedings of the Pennsylvania Academy of Science, vol. 38, p. 84-89.

Beamish, F. W. H.
1964. Respiration of fishes with special emphasis on standard oxygen consumption; II, Influence of weight and temperature on respiration of several species. Canadian Journal of Zoology, vol. 42, p. 177-188.

Cheah, K. S.
1967. The oxidase systems of Moniezia expansa (Cestoda). Journal of Comparative Biochemistry and Physiology, vol. 23, p. 277-302.

Derse, Philip H., and F. M. Strong.
1963. Toxicity of antimycin to fish. Nature, vol. 200, no. 4906, p. 600-601.

Hiltebran, Robert C.
1965. Oxidation of succinate by bluegill liver mitochondria. Transactions of the Illinois Academy of Science, vol. 58, no. 3, p. 176-182.
1967. Oxidation of alpha-ketoglutarate by bluegill liver mitochondria. Transactions of the Illinois Academy of Science, vol. 60, no. 3, p. 244-249.

Hunn, Joseph B., Richard A. Schoettger, and Everett W. Whealdon.
1968. Observations on the handling and maintenance of bioassay fish. The Progressive Fish-Culturist, vol. 30, no. 3, p. 164-167.

Lennon, Robert E., and Charles R. Walker.
1964. Laboratories and methods for screening fish-control chemicals. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 1, (Bureau Circular 185). 15 p.

Rajagopalan, K. V., I. Fridovich, and P. Handler.
1962. Hepatic aldehyde oxidase. Journal of Biological Chemistry, vol. 237, p. 922-925.

Rieske, John S.
1967. Antimycin A. Volume 1. Mechanisms of action. p. 542-584. In Antibiotics, Editors David Gottlieb and Paul D. Shaw. Springer-Verlag, Berlin.

Rieske, J. S., and W. S. Zaugg.
1962. The inhibition by antimycin A of the cleavage of one of the complexes of the respiratory chain. Biochemistry and Biophysics Research Communications, vol. 8, p. 421-431.

Rieske, John S., Harold Baum, C. D. Stoner, and S. H. Lipton.
1967. On the antimycin-sensitive clearage of complex III of the mitochondrial respiratory chain. Journal of Biological Chemistry, vol. 242, no. 21, p. 4854-4866.

Ritter, P. O., and F. M. Strong.
1966. Residues in tissues of fish killed by antimycin. Journal of Agricultural Food Chemistry, vol. 14, no. 4, p. 403-407.

Schoettger, Richard A., and Gerald E. Svendsen.
1968. Physiology of toxicants, p. 121. In Progress in Sport Fishery Research, 1967. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 64.

Steen, Johan B., and A. Kruysse.
1964. The respiratory function of teleostean gills. Journal of Comparative Biochemistry and Physiology, vol. 12, p. 127-142.

Steen J. B., and T. Berg.
1966. The gills of two species of haemoglobin-free fishes compared to those of other teleosts — with a note on severe anaemia in an eel. Journal of Comparative Biochemistry and Physiology, vol. 18, p. 517-526.

Strong, F. M.
1958. Topics in microbial chemistry. Antimycin, Coenzyme A, Kinetin and Kinins. John Wiley & Sons, New York. 166 p.

Takemori, S., and T. E. King.
1964. Coenzyme Q: reversal of inhibition of succinate cytochrome c reductase by lipophilic compounds. Science, vol. 144, p. 852.

Umbreit, W. W., R. H. Burris, and J. F. Stauffer.
1964. Manometric techniques. Burgress Publishing Company, Minneapolis, Minn. 305 p.

Walker, Charles R., Robert E. Lennon, and Bernard L. Berger.
1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 2 (Bureau Circular 186). 18 p.

INVESTIGATIONS IN FISH CONTROL

40. A Resume on Field Applications of Antimycin A to Control Fish

By Robert E. Lennon and Bernard L. Berger

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A RESUME ON FIELD APPLICATIONS OF ANTIMYCIN A TO CONTROL FISH

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ABSTRACT.--Antimycin A, a fish toxicant, has had more than 50 applications to control fish in the field. It has been used for partial reclamations, and as a general or selective toxicant. It is effective against fish in fresh and marine waters, in acid and alkaline waters, in cold and warm waters, and in flowing and static waters. The formulations contribute no color or odor to water and do not repel fish. The toxic action, respiratory inhibition, is irreversible in most fishes. Fish-killing concentrations are harmless to most aquatic invertebrates and to higher vertebrates. Highlights of the field applications are presented.

The newest tool in fish management is antimycin A, an antibiotic produced by molds of the genus Streptomyces. Scientists at the University of Wisconsin isolated it in 1945 and thereafter tested it for years against fungi which destroy crops. During experiments in 1963, the potential of antimycin A as a fish toxicant was recognized. A use patent was obtained by the Wisconsin Alumni Research Foundation.

The antibiotic was tested exhaustively by the Bureau's Fish Control Laboratories against many species of fish in waters of diverse qualities. Concurrently, private laboratories investigated its toxicity to mice, rats, rabbits, guinea pigs, dogs, lambs, quails, pigeons, chickens, pheasants, and mallard ducks.

Antimycin is a powerful fish toxicant because minute quantities inhibit the transport of electrons at a very specific site in the respiratory system. The amounts of antimycin which kill fish are harmless to mammals and birds.

Antimycin is effective against fish, eggs to adults, in fresh and salt water, in acid and alkaline water, in clear and turbid water, and in cold and warm water. The toxic action on most species is irreversible. It evokes no spectacular response from fish. They die slowly and exhibit no frenzied activity. The toxicant contributes no odor or color to the water, and it seems to be undetected by fish. It therefore does not repel fish, which is a very important advantage in reclaiming lakes and streams.

Although stable when dry, antimycin is nonpersistent and degrades rapidly in water. It is susceptible to detoxification by small quantities of potassium permanganate.

Several formulations of antimycin were employed in experiments in the laboratory and field. A liquid formulation was used in most of the laboratory trials and in streams. Three dry formulations called Fintrol-5, Fintrol-15, and Fintrol-30 were tested in the laboratory and in lakes and ponds. These novel preparations consist of antimycin coated on sand particles in such a way that the toxicant is released into the water as the sand sinks to the bottom. Fintrol-5 gives up its antimycin evenly within the first 5 feet of depth, Fintrol-15 within 15 feet, and Fintrol-30 within 30 feet of depth.

The Governments of the United States and Canada registered antimycin A as a fish toxicant in 1966, based on the far reaching research which demonstrates that it is effective on fish but relatively harmless to water weeds, aquatic insects, frogs, salamanders, turtles, mammals, and birds. Ayerst Laboratories has obtained registration of Fintrol-5, a shallow-water formulation of antimycin on sand. Liquid and deep-water formulations will become available to fishery managers in the near future.

FIELD TRIALS

A review of more than 50 applications of antimycin in lakes and streams in 19 States and in Canada and Guatemala indicates that it is advantageously flexible as well as effective against fish in a variety of uses and situations (tables 1-4). This summary highlights only a few of its potentials; ingenuity of fish managers may disclose other uses of the antibiotic.

Two basic criteria are involved in a lethal dose of toxicant: the concentration in water, expressed here in parts per billion (ppb), and the duration of exposure of fish to the toxicant. Although frequently overlooked, exposure is critical and must be correlated with concentration to obtain a lethal dose. Axiomatically, a low concentration must be accompanied by a long exposure to achieve a lethal dose. Conversely, a high concentration plus short exposure may equal a lethal dose. These relations must be observed with greater care when applying a toxicant to flowing waters.

Antimycin in fresh and salt waters

Most experimentation with antimycin was in fresh water and against freshwater fishes. Results are presented in later sections.

TABLE 1.--List of fishes exposed to antimycin

Abbreviation	Common name	Scientific name
Am Brk Ly	American brook lamprey	Lampetra lamottei
Am El	American eel	Anguilla rostrata
Am St	American smelt	Osmerus mordax
An	Anchovies	Engraulidae--family
At Sm	Atlantic salmon	Salmo salar
Bc Sh	Blackchin shiner	Notropis heterodon
Bf	Bowfin	Amia calva
Bf Hy	Buffalo hybrid	
Bg SnFh	Bluegill sunfish	Lepomis macrochirus
Bk BlHd	Black bullhead	Ictalurus melas
Blk Cr	Black crappie	Pomoxis nigromaculatus
Brk Sb	Brook stickleback	Eucalia inconstans
Brk Ss	Brook silverside	Labidesthes sicculus
Brk Tr	Brook trout	Salvelinus fontinalis
Bl CtFh	Blue catfish	Ictalurus furcatus
Bl Sk	Bridgelip sucker	Catostomus columbianus
Bm Bf	Bigmouth buffalo	Ictiobus cyprinellus
Bn Dc	Blacknose dace	Rhinichthys atratulus
Bn Mw	Bluntnose minnow	Pimephales notatus
Bn Sh	Blacknose shiner	Notropis heterolepis
Br BlHd	Brown bullhead	Ictalurus nebulosus
Br Tr	Brown trout	Salmo trutta
Bt RdHs	Blacktail redhorse	Moxostoma poecilurum
Ch CtFh	Channel catfish	Ictalurus punctatus
Chm	Chiselmouth	Acrocheilus alutaceus
Ch Pl	Chain pickerel	Esox niger
Cm Jk	Chiselmouth jack	Coregonus oregonus
Cm Sh	Common shiner	Notropis cornutus
Cn MdMw	Central mudminnow	Umbra limi
Co Sm	Coho salmon	Oncorhynchus kisutch
Cp	Carp	Cyprinus carpio
Cr Cb	Creek chub	Semotilus atromaculatus
Ct Tr	Cutthroat trout	Salmo clarki
Dy Vn Tr	Dolly Varden	Salvelinus malma
Fh CtFh	Flathead catfish	Pylodictus olivaris
Fh Mw	Fathead minnow	Pimephales promelas
Fl Fh	Fallfish	Semotilus corporalis
Fl SnFh	Flier sunfish	Centrarchus macropterus
Ft Dr	Fantail darter	Etheostoma flabellare
Fw Dm	Freshwater drum	Aplodinotus grunniens
Gd Sh	Golden shiner	Notemigonus crysoleucas
Gf	Goldfish	Carassius auratus
Gr Cp	Grass carp	Ctenopharyngoden idellus
Gr Pl	Grass pickerel	Esox americanus vermiculatus
Gr SnFh	Green sunfish	Lepomis cyannellus
Gz Sd	Gizzard shad	Dorosoma cepedianum
Hy SnFh	Hybrid sunfish	
Ia Dr	Iowa darter	Etheostoma exile
Jh Dr	Johnny darter	Etheostoma nigrum
Jk	Jacks	Carangidae--family
Kk Sm	Kokanee salmon	Oncorhynchus nerka
Le SnFh	Longear sunfish	Lepomis megalotis
Lk Cb Sk	Lake chub sucker	Erimyzon sucetta
Lk Tr	Lake trout	Salvelinus namaycush
Lm Bs	Largemouth bass	Micropterus salmoides
Ln Dc	Longnose dace	Rhinichthys cataractae
Ln Gr	Longnose gar	Lepisosteus osseus
Ln KlFh	Longnose killfish	Fundulus similis
Ln Sh	Longnose shiner	Notropis longirostris
Ln Sk	Longnose sucker	Catostomus catostomus
Ls Sk	Largescale sucker	Catostomus macrocheilus
Mq Fh	Mosquito fish	Gambusia affinis
Mt Sp	Mottled sculpin	Cottus bairdi
No Hg Sk	Northern hog sucker	Hypentelium nigricans
No Pk	Northern pike	Esox lucius

TABLE 1.--List of fishes exposed to antimycin--Continued

Abbreviation	Common name	Scientific name
No Rb Dc	Northern redbelly dace	Chrosomus eos
No RdHs	Northern redhorse	Moxostoma macrolepidotum
No SqFh	Northern squawfish	Ptychocheilus oregonensis
Os Sf	Orangespotted sunfish	Lepomis humilis
Qb	Quillback	Carpiodes cyprinus
Pd Fh	Paddlefish	Polyodon spathula
Pm	Peamouth	Mylocheilus caurinus
Pp	Pompano	Carangidae--family
Ps SnFh	Pumpkinseed	Lepomis gibbosus
Rb SnFh	Redbreast sunfish	Lepomis auritus
Rb Tr	Rainbow trout	Salmo gairdneri
Re SnFh	Redear sunfish	Lepomis microlophus
Rk Bs	Rock bass	Ambloplites rupestris
Rs Sh	Redside shiner	Richardsonius balteatus
S CtFh	Sea catfish	Galeichthys felis
Sm Bf	Smallmouth buffalo	Ictiobus bubalus
Sm Bs	Smallmouth bass	Micropterus dolomieui
Sh Mw	Sheepshead minnow	Cyprinodon variegatus
Sh TpMw	Starhead topminnow	Fundulus notti
Sn Gr	Shortnose gar	Lepisosteus platostomus
S Ly	Sea lamprey	Petromyzon marinus
So Rb Dc	Southern redbelly dace	Chrosomus erythrogaster
Sp Gr	Spotted gar	Lepisosteus oculatus
Sp SnFh	Spotted sunfish	Lepomis punctatus
Sp Sk	Spotted sucker	Minytrema melanops
Spt	Spot	Leiostomus xanthurus
St Rr	Stoneroller	Campostoma anomalum
Tl Sh	Taillight shiner	Notropis maculatus
Tp	Tilapia	Tilapia mossambica
Tp Mt	Tadpole madtom	Noturus gyrinus
Tr Sp	Torrent sculpin	Cottus rhotheus
Wd Sh	Weed shiner	Notropis texanus
We	Walleye	Stizostedion vitreum vitreum
Wm Bs	Warmouth bass	Chaenobryttus gulosus
Wt Bs	White bass	Roccus chrysops
Wt Cr	White crappie	Pomoxis annularis
Wt CtFh	White catfish	Ictalurus catus
Wt Sk	White sucker	Catostomus commersoni
Yw BlHd	Yellow bullhead	Ictalurus natalis
Yw Ph	Yellow perch	Perca flavescens

The Bureau of Commercial Fisheries has exposed marine fish to antimycin at the Biological Laboratory, Gulf Breeze, Fla. and in a tidal basin near Fort Desoto, Fla. Thirty-eight species in the basin received a 6-hour--a tidal cycle--exposure to 7 ppb of antimycin. It killed the more sensitive species such as anchovies, triggerfish, flounders, and rays. The more resistant catfishes, common jack, and redfish did not all succumb during this brief exposure.

Antimycin in acid water

Antimycin persists longer in acid water than in alkaline water, and the application rate may be reduced.

The acid-water trials included trout ponds in northern New Hampshire and Quebec and panfish ponds in northern Wisconsin and Georgia. In New Hampshire, 1 ppb of antimycin eliminated 12 species of fish from a pond within 72 hours. Those killed included Atlantic salmon, chain pickerel, minnows, white sucker, pumpkinseeds, largemouth bass, and yellow perch. A few bluegills and smallmouth bass and all brown bullheads survived. In another pond, 12 ppb quickly killed all species except bullheads within 48 hours, the antimycin persisting for 2 weeks at toxic level.

In Quebec, 5 ppb of antimycin eradicated 13 species from Beauty Lake. The principal target fish in this 44-acre brook-trout lake were northern pike, minnows, suckers, pumpkinseeds, and yellow perch.

The trials in acid waters in eastern U.S. also confirmed the results of tests in the laboratory, demonstrating that catfishes, such as bullheads, are not harmed by small concentrations of antimycin. The complete survival of catfishes was thus expected in subsequent field trials which involved less than 25 ppb of antimycin.

Antimycin in alkaline water

Most field applications of antimycin have been in alkaline waters which ranged from pH 7.0 to 9.3. They confirmed that the toxicant is pH-sensitive, with the more rapid degradation occurring at higher pH's. Whereas rapid degradation can be exploited to great advantage in many situations where there are multiple uses of the lakes or streams, the duration of exposure to an effective concentration of toxicant must be calculated carefully to secure a lethal result on the target fish. In general greater concentrations of toxicant are required at pH 8.5 or higher to offset rapid degradation.

In 35-acre Lake Katrine near Madison, Wis., 7.5 ppb of antimycin caused a complete kill of carp and fathead minnows at pH 8.2 and 56° F. In 63-acre Parker Lake, Wis.,

TABLE 2.--Sites of field applications with data on water quality

Trial	Date	Location	Area or volume	Temperature (°F.)	pH	Hardness (ppm)	Alkalinity (ppm)
1......	10/63	Research pond, Delafield, Wis.	0.75 a.	65	8.5	213	210
2-4....	9/64	Hatchery ponds, NFH, Berlin, N.H.					
		Pond No. 1	0.45 a.-ft.	53	7.0	10	14
		Pond No. 2	1.16 a.-ft.	53	7.2	10	13
		Pond No. 3	0.55 a.-ft.	53	7.2	10	15
5......	9/64	Pond, Fort Benning, Ga.	0.23 a.	83	6.9	9	12
6-8....	9/64	Hatchery ponds, NFH, Cape Vincent, N.Y.					
		Pond No. 1	1.0 a.	53	8.1	153	110
		Pond No. 2	1.0 a.	53	7.9	159	118
		Pond No. 3	1.0 a.	53	8.1	155	118
9-11...	3/65	Research ponds, Stuttgart, Ark.					
		Pond No. 1	0.25 a.	63	8.0	59	79
		Pond No. 2	0.25 a.	63	8.1	58	74
		Pond No. 3	0.25 a.	63	8.2	55	75
12-13..	5/65	Natural ponds, NWR, Valentine, Nebr.					
		Pond No. 1	1.8 a.	65	8.4	107	155
		Pond No. 2	0.5 a.	70	7.9	142	258
14.....	8/65	Sidie Hollow Creek, Viroqua, Wis.	1.8 cfs.	53	8.3	240	--
15.....	10/65	Veteran's Memorial Pond, West Salem, Wis.	5.0 a.	56	7.8	223	188
16.....	10/65	Lake Creek Lake, NFH, Saratoga, Wyo.	2.0 a.	48	7.7	237	184
17-18..	12/65	Research ponds, Stuttgart, Ark.					
		Pond No. 1	22 a.	53	8.6	112	110
		Pond No. 2	0.25 a.	50	8.6	120	110
19.....	2/66	Lake Atitlan, Guatemala	2.5 a.	72	7.7	--	163
20.....	5/66	Barney Lake, Madison, Wis.	34 a.	68	8.5	26	--
21.....	5/66	Katrine Lake, Madison, Wis.	34 a.	63	8.4	32	25
22.....	7/66	Alto Creek, Fox Lake, Wis.	4.9 cfs.	60	7.7	hard	--
23.....	7/66	Drew Creek, Fox Lake, Wis.	7.2 cfs.	60	7.8	hard	--
24.....	8/66	Unknown Lake, Oneida County, Wis.	--	72	5.2	4	--
25.....	8/66	Perch Lake, Wis.	--	74	neutral	soft	--
26.....	8/66	Harriet Lake, Wis.	--	76	neutral	soft	--
27-29..	8/66	Farm ponds,					
		Pond No. 1	0.94 a.	82	8.5	20	22
		Pond No. 2	1.26 a.	86	8.5	20	19
		Pond No. 3	1.17 a.	86	8.5	14	20
30.....	9/66	Mullet River, Plymouth, Wis.	10.2 cfs.	70	8.3	210	--
31.....	10/66	Barney Lake, Madison, Wis.	35 a.	56	8.2	--	35
32.....	10/66	Parker Lake, Adams County, Wis.	63 a.	48	8.2	197	--
33.....	10/66	Beauty Lake, Quebec	50 a.	60	7.0	60	75
34.....	11/66	Rathbone Creek, Cataract, Wis.	10.7 cfs.	48	7.5	24	--
35.....	1967	Catfish pond, Baton Rouge, La.	1.2 a.	74	7.2	--	--
36.....	5/67	Catfish rearing ponds, Greenwood, Mo.	28 a.	64	7.5	--	--
37.....	6/67	Backbone Lake, Delaware County, Ia.	125 a.	68	8.3	--	--
38.....	6/67	Catfish rearing ponds, Leland, Miss.	10-15 a.	78	high	50	--

TABLE 2.--Sites of field applications with data on water quality--Continued

Trial	Date	Location	Area or volume	Temperature (°F.)	pH	Hardness (ppm)	Alkalinity (ppm)
39.....	6/67	Westfield Creek, Westfield, Wis.	22 cfs.	77	8.8	--	170
40.....	7/67	Research pond, Stuttgart, Ark.	0.3 cfs.	82	8.3	--	--
41.....	8/67	Pleasant Lake, Minn.	20 a.	67	8.0	--	--
42.....	8/67	Delbert Gnegy Pond, Oakland, Md.	0.43 a.	65	8.6	80	--
43.....	9/67	Golf Course Pond, Shaw Air Force Base, S.C.	3.9 a.	79	7.2	15	16
44.....	9/67	Tarrant Lake, Cambria, Wis.	0.5 a.-ft.	50	7.7	--	270
45.....	9/67	Hatchery ponds, Avoca, Ind.	0.4 a.	72	7.5	--	--
46.....	9/67	Turquoise Lake, Leadville, Colo.	1250 a.-ft.	56	8.0	53 TDS	19
47.....	9/67	Farm ponds, Warm Springs, Ga.	0.4-1.0 a.	75	7.3	15	14
48.....	10/67	Bigfoot Beach Lagoon, Lake Geneva, Wis.	7.5 a.	54	7.9	--	260
49.....	10/67	Gold-tailing ponds, Powder River Rehabilitation, Sumter, Oreg.	0.02-0.34 a.	55	7.6	soft	--
50.....	10/67	Victory Pond, Fort Benning, Ga.	26 a.	70	7.0	--	--
51.....	10/67	Barney Lake, Madison, Wis.	34 a.	57	8.7	--	35
52.....	10/67	Whitewater Lake, NWR, Valentine, Nebr.	490 a.	62	9.3	204	310

10 ppb wiped out carp, pumpkinseed, bluegill, and yellow perch at pH 8.2 and 48°. The application of 10 ppb of toxicant in 34-acre Barney Lake, Madison, Wis., killed all carp at pH 8.7 and 57°. On the other hand, 7.3 ppb of antimycin in Whitewater Lake, Valentine, Nebr., at pH 9.3 degraded before most of the carp and other fish had sufficient exposure to kill them. Bioassays indicated that 12 to 15 ppb of toxicant were needed to produce a kill at the high pH.

Antimycin in cold and warm waters

In general, the response of fish to antimycin is slower in cold water than in warm water because of reduced metabolic activity. Thus, to insure adequate duration of exposure before degradation of the compound, a higher concentration of antimycin may be required when temperatures are below 50° F.

The performance of antimycin in cold water was observed best in simulated field tests in 1/100-acre pools at the Fish Control Laboratory, La Crosse, Wis. Brown trout, carp, bluegills, and largemouth bass were killed by 5 ppb within 2 to 7 days at pH 7.4 to 8.2 and 38° to 42° F. Other tests under ice in 1,000-gallon vinyl pools demonstrated that antimycin at 5 ppb killed rainbow trout, brown trout, small carp, bluegills, and longear sunfish. Survivors included large goldfish, large carp, fathead minnows, and pumkinseeds. Thus, a greater concentration of toxicant would have been needed to kill all fish. Other things being equal, lower concentrations of toxicant and more rapid response of fish can be expected in water over 60° F.

Nonrepellency of antimycin

The formulations of antimycin coated on sand cause no color or odor when applied to water. Fish make no attempt to avoid contact with treated water--a great advantage when reclaiming springfed lakes and barrier-free streams.

TABLE 3.--Results with antimycin as a general toxicant

Trial site	Objective	Species (refer to table 1)	Anti-mycin (ppb)	Formu-lation	Results (kill)
pH below 7.5--temp. 60°F. or above					
5.....	Determine effect on species present	GrPl, GdSh, LnSh, TlSh, MqF, BkSb, AmEl, FlSf, RbSf, GrSf, OsSf	0.6	Technical	Total except bluegill, warmouth, and catfishes.
33.....	Eradicate northern pike and stunted sunfish	RbTr, BkTr, AmSt, NoPk, GdSh, BcSh, BnSh, WdSh, BlSk, BlCtF, WtB, SmBs, JhDr	5.0	Fintrol 5, 15, 30 & liquid	Total except bullheads.
55.....	Determine effect of low concentration on species present	GzSd, GrPl, GdSh, BtRdH, AmEl, FlSf, BgSf, ReSf, WtCr	0.9	Liquid	Total on GzSd; partial on all other species.
53.....	Determine effect of low concentration on species present	ChPl, TlSh, ShTpMw, BgSf, ReSf, SpSf, LmBs, BrSs	0.4	Liquid	Partial on all species.
pH below 7.5--temp. below 60°F.					
54.....	Determine effect of low concentration on species present	ChPl, LkChSk, AmEl, BgSf, ReSf, LmBs, WtCr	1.5	Liquid	Partial on all species.
2-4....	Determine effect on species present	RbTr, AtSm, BrTr, BkTr, ChPl, GdSh, CmSh, NoSqF, RsSh, CrCb, BlSk, BlCtF, WtB, FlSf, PsSf, OsSf, SmBs	0.13	Fintrol	Total only on common shiner.
	"	"	1.22	Fintrol	Total except bluegill, largemouth bass, and brown bullhead.
	"	"	12.1	Fintrol	Total except brown bullhead.
34....	Eradicate white sucker	AmBkLy, BkTr, CnMdMn, BlSk, ShMw, HybSf	7.5	Fintrol-5 & liquid	99 percent.
pH 7.5-8.5--temp. above 60°F.					
9-11...	Determine effect on species present	RbTr, Cp, GdSh, LkCbSk, NoHgSk, SmBf, BkBh, MqF, AmEl, FlSf, OsSf, LeSf	5.0	Fintrol-5	Total except catfishes and warmouth.
	"	"	7.5	Fintrol-5	Total except catfishes.
	"	"	10.0	Fintrol-5	Total except catfishes.
45.....	Determine effect on species present	SpGr, Bf, GzSd, Chm, Cp, FlF, BlSk, LkCbSk, NoRdH, WtCtF, BkBh, MqF, AmEl, WtB, FlSf, OsSf, SmBs	5.0	Fintrol-5	Total except spotted gar, channel catfish, and bullheads.
30.....	Eradicate carp from a stream	CnMn, NoPk, Cp, GdSh, RsSh, BlSk, LnKf, AmEl, FlSf, OsSf, LeSf	7.5	Fintrol-5 & liquid	99 percent.
12.....	Eradicate all fish	NoPk, Cp, BnMw, SCtF, AmEl, FlSf, OsSf, BgSf, SmBs	10.0	Fintrol-5	Total except northern pike, bullhead, largemouth bass, and black crappie.
13.....	Eradicate all fish	NoPk, Cp, BnMw, SCtF, FlSf, OsSf, SmBs	10.0	Fintrol-5	Total except black bullhead.
19.....	Eradicate largemouth bass from a grebe sanctuary	OsSf, LeSf	10.0	Fintrol-5	Total on largemouth bass and black crappie.
pH 7.5-8.5--temp. below 60°F.					
6-8....	Determine effect on species present	Bf, RbTr, BrTr, BkTr, Chm, Cp, GdSh, BlSk, BlCtF, ShTpM, LnKf, AmEl, WtB, FlSf, PsSf, LeSf, SmBs, LgBs, IaDr	1.0	Fintrol	Total only on trouts, minnows, and white sucker.
	"	"	3.1	Fintrol	Total except bowfin, bullheads, pumpkinseed, and largemouth bass.
	"	"	10.0	Fintrol	Total except bowfin and bullheads.
46....	Eradicate white and longnose suckers	KkSm, ClTr, RbTr, BrTr, BkTr, SoRbD, Cb, BlSk	3.5	Fintrol-5 & liquid	65 percent.
48....	Eradicate carp	Cp	5.0	Fintrol-5	Total.

TABLE 3.--Results with antimycin as a general toxicant--Continued

Trial site	Objective	Species (refer to table 1)	Antimycin (ppb)	Formulation	Results (kill)
pH 7.5-8.5--temp. below 60°F.--Continued					
49.....	Eradicate squawfish, suckers, shiners	CmJk, RbTr, BkTr, DyVnTr, Pm, NoSqF, BnD, LnD, YwPh	7.5	Fintrol-5	Total on target species.
15.....	Eradicate total except catfish	RbTr, BrTr, CnMdMn, NoPk, Cp, GdSh, BnMw, BlSk, SCtF, BkBh, AmEl, WtB, FlSf	10.0	Fintrol-5	Total except channel catfish and bullheads.
16.....	Eradicate all fish from hatchery water supply	BkTr, BnMw, RsSh, ReSf	10.0	Fintrol-5	Total.
22.....	Eradicate carp in stream	Bf, CnMdMn, NoPk, Cp, NoSqF, BnD, BlSk, ShMw, FlSf	10.0	Fintrol & liquid	Total
23.....	Eradicate carp in stream	Cp	10.0	Fintrol & liquid	99 percent.
31.....	Eradicate carp	Cp, BnMw	7.5	Fintrol-5	Total.
32.....	Eradicate all fish	RbTr, Cp, WtB, FlSf, SmBs	10.0	Fintrol 5, 10, 15	Total.
14.....	Eradicate all fish	RbTr, BrTr, StRr, NoSqF, BnD, RsSh, BlSk, LsSk, ShMw, HybSf, JhDr	15.0	Technical	Total.
44.....	Eradicate carp and black bullhead	Cp, SCtF	150.0	Fintrol & liquid	Total.
pH above 8.5--temp. 60°F. or above					
52.....	Eradicate carp	ChPl, LsSk, ChCtF	7.3	Fintrol-5	Small kill.
1......	Delineate effect on species present	AmBkLy, Bf, RbTr, NoPk, Gf, Cp, BlSk, SCtF, WtCtF, AmEl, WtB, FlSf, GrSf, OsSf, LeSf, SmBs, LmBs, IaDr	10.0	Technical	Total except longnose gar, bowfin, and black bullhead.
39.....	Eradicate carp and white sucker	AmBkLy, RbTr, Cp, WdSh, BnMw, NoSqF, RsSh, BlSk, ShMw, WtB, FlSf, OsSf, SpSf, HybSf, JhDr	10.0	Fintrol & liquid	99 percent.
pH above 8.5--temp. below 60°F.					
17.....	Eradicate all fish	Pf, GzSd, Gf, Cp, NoHgSk, BtRdH, SCtF, WtCtF, BkBh, YwBh, BrBh, ChCtF, MqF, AmEl, RkB, FlSf, OsSf, LeSf	10.0	Fintrol-5	Total except for 7 species of catfish.
18.....	Eradicate all fish	Pf, Cp, GdSh, LkCbSk, NoHgSk, BkBh, MqF, AmEl, RkB, FlSf, OsSf	10.0	Fintrol-5	Total on scale fish.
51.....	Eradicate carp	Cp	10.0	Fintrol & liquid	Total.

The clear and colorless water of Lake Creek Lake at Saratoga, Wyo., NFH afforded a fine opportunity to test the nonrepellency of antimycin. The 1.8-acre lake is entirely spring fed, and its discharge of 3 cfs supplies the hatchery with water. Our objective was to rid the lake of fish which might serve as vectors for diseases. Previous attempts with chlorine and rotenone failed to eradicate the fish because they fled into bottom springs and other springs beneath undercut banks.

The spring flow affected exchange of water in the basin of the lake every 27 hours: thus maintenance of adequate exposure to a toxicant was most critical. Therefore, we maintained at least 10 ppb of antimycin in the lake for a minimum of 8 hours. A seed spreader achieved even distribution of the sand formulation which sank readily into the bottom springs.

The fish were clearly seen to exhibit no alarm or tendency to escape from the treated water. We first observed toxic effects 2 hours after applying antimycin: Dense schools of large northern creek chubs began to break up and seemed to lose their orientation with respect to schooling. None sought relief in spring flows, and most were dead within 24 hours.

TABLE 4.--Results with antimycin as a selective toxicant

Trial site	Objective	Species (refer to table 1)	Antimycin (ppb)	Formulation	Results (kill)
pH below 7.5--temp. 60°F. or above					
24.....	Eradicate yellow perch and small bluegill	NoPk, WdSh, SCtF, LnKf, FlSf, PsSf	1.0-2.0	Fintrol-5	Total on yellow perch and partial on bluegill.
25.....	Eradicate yellow perch	NoPk, GdSh, WdSh, SCtF, LnKf, PsSf	0.5	Fintrol-5	Total on yellow perch.
26.....	Eradicate yellow perch	NoPk, GdSh, WdSh, SCtF, LnKf, PsSf	0.5	Fintrol-5	Total on yellow perch.
47.....	Thinning sunfish in bass-bluegill pond	GdSh, BlCtF, BkBh, FhCtF, BkSb, RkB, FlSf, RbSf, OsSf, BgSf	0.4-1.0	Fintrol-5	0.4 to 0.6 ppb gave excellent control.
35.....	Eradicate scale fish from catfish pond	CmSh, BkBh, FhCtF, AmEl, OsSf	3.0	Fintrol-5	Total on scalefishes.
50.....	Eradicate scale fish from catfish pond	GdSh, BkBh, MqF, FlSf, OsSf	3.6	Fintrol-5	Total except channel catfish, bullhead, and gambusia.
pH 7.5-8.5--temp. above 60°F.					
21.....	Kill carp eggs in pond	Cp	4.0	Fintrol-5	Total on eggs in area.
37.....	Eradicate adult carp in spawning bays	Cp, FlSf, OsSf, LeSf	50.0	Fintrol-5	Partial control.
36.....	Eradicate scale fish from catfish pond	GdSh, AmEl, FlSf, OsSf	5.0	Fintrol-5	Total on golden shiner, bass, bluegill, and green sunfish.
43.....	Eradicate scale fish from catfish pond	BkBh, FlSf	5.0	Fintrol-5	Total on bluegills.
41......	Eradicate minnows from trout lake	RbTr, SoRbD, GdSh, BnSh, WdSh, NoSqF, BlSk, ShMw	5.0	Fintrol-5, -15	Total on minnows.
pH above 8.5--temp. 60°F or above					
20.....	Kill carp eggs	Cp	2.0	Technical	Partial kill on eggs.
38.....	Eradicate scale fish from catfish pond	GdSh, BkBh, AmEl	4.0	Fintrol-5	Total on scalefish.
27-29..	Eradicate scale fish from catfish pond	GdSh, MqF, AmEl, FlSf	5.0	Fintrol-5	99 percent scalefish.
	"	"	7.5	Fintrol-5	99 percent scalefish.
	"	"	10.0	Fintrol-5	Total on scalefish.
42.....	Eradicate stunted sunfish	BkBh, YwBh	7.5	Fintrol-5	Total.
40.....	Reduce gizzard shad in spot treatments	GzSd, AmEl	1.5-6.5 ppm	Fintrol & liquid	Reduced population.

Brook trout in the lake ranging from one-third ounce to 6.6 pounds, easily observed throughout the reclamation, began to die 3 hours after exposure. None attempted to escape from the lake via the outlet or to move into the springs. Numerous fathead minnows and the small numbers of Iowa darters responded similarly to the creek chubs and brook trout. Antimycin killed all the fish and was flushed from the basin by spring flows within 48 hours.

Westfield Creek in south-central Wisconsin also provided an opportunity to observe the behavior of fish under exposure to antimycin. Here the principal target fish was carp, a species particularly adept at escaping exposure to toxicants which contain repelling ingredients. We treated a 3.5-mile portion of Westfield Creek which had an average flow of 17.2 cfs, pH 8.8, total alkalinity of 170 ppm, and a temperature of 77° F. The water was clear and colorless, and had an average velocity of 0.5 feet per second. Antimycin in liquid formulation was maintained at 10 ppb for 10 hours by drip stations and spray pumps throughout the 3.5 miles of stream.

None of the numerous fish present attempted to avoid contact with the toxicant by fleeing downstream. Rather, fish gradually drifted downstream hours later when the antimycin had rendered them incapable of maintaining positions in the current. Fantail and johnny darters began to drift downstream and to die within 2 hours, followed soon by fathead and bluntnose minnows and blacknose dace, and later by common shiners, creek chubs, white suckers, bluegills, pumpkinseeds, and largemouth bass. Distressed and dying carp by the thousands began to drift involuntarily downstream after 8 hours. Brook lampreys and slimy sculpins were the last species to exhibit distress and die.

Most of the carp were dead within 24 hours. The few living specimens, all in distress, perished within 48 hours. None recovered from the toxicant, although it had flushed out of the stream hours before. Intensive electrofishing on 1.5 miles of the stream a week later failed to turn up any carp, suckers, or panfish. A few small minnows, a brook stickleback, and several brook lampreys were the only living fish found. The reclamation was an unqualified success.

Antimycin in partial reclamations

Antimycin is better suited to partial reclamation of lakes than any toxicant heretofore available. Its advantages here are (1) the lack of repellency and (2) the effectiveness on all life stages of fish, eggs through adults.

Two soft-water, high-pH lakes near Madison, Wis., were used in early experiments. Treatments of shoreline spawning areas and a bay in Lake Barney with 2 ppb of antimycin failed to kill all carp eggs. But concurrently the treatment of a spawning bay in nearby Lake Katrine with 4 ppb of antimycin resulted in complete mortality of carp eggs. Eggs in control areas of the lake hatched, and fry were easily collected; only dead eggs were found in the treated bay, and no fry could be collected.

Large numbers of carp congregated on spawning sites in Backbone Lake in Iowa were exposed to 50 ppb of antimycin. We assumed that this concentration would be lethal to both adults and spawn if they remained exposed for 8 hours or more. The staked sites ranged from a fourth of an acre to 5 acres in area, and the toxicant was applied at night while fish were moving into the spawning areas. Within hours after the application, a brief but violent storm brought 2 inches of rain and the lake rose 3 inches in level, causing a dilution of toxicant and cessation of spawning activity. The trial demonstrated, however, that carp on the spawning sites or moving into them were not repelled by the antimycin, and many succumbed.

Another approach to partial reclamation was made in a 22-acre lake at the Fish Farming Experimental Station at Stuttgart, Ark. Here we wanted to reduce large numbers of gizzard shad which at times congregated at the inlet to the pond, but without harming the channel catfish in the pond. The first treatment involved a 6-minute titration of 50 cc of liquid antimycin into the 140-gpm inlet flow. The shad dispersed after 1.5 minutes, but they had already sustained a lethal exposure and large numbers died. Two days later a 1.5-minute titration of 50 cc of toxicant in the same flow killed large numbers of shad which had migrated into the vicinity of the inlet. A third application with 100 cc of antimycin was made 2 days later to kill a new congregation of shad, and on this occasion some green sunfish perished. The treatments were effective and economical in reducing the numbers of gizzard shad without harming channel catfish.

McGrath Lake in northern Wisconsin afforded an opportunity to attempt partial control of stunted panfish. The lake had a pH of 5.2, total hardness of 4 ppm, and a temperature of 70° F. Antimycin applied to one of the well-defined bays at 1 ppb killed all of the stunted yellow perch and 50 percent of the stunted bluegills. Another bay received 2 ppb of the toxicant, and all perch and bluegills were eliminated.

Antimycin as a selective toxicant

Fish species differ in their sensitivity to antimycin, thus offering an opportunity in many situations to practice selective control of target fish. Stunted yellow perch, for example, were the targets for selective kills in Harriet and Perch lakes in northern Wisconsin. The waters of both lakes at the time were soft, acid, and warm. As intended, antimycin at 0.5 ppb killed all of the stunted perch without harm to northern pike, bluntnose minnows, bullheads, largemouth bass, and smallmouth bass.

Worthwhile reductions of overabundant minnows, sunfish, and crappies have also been accomplished in soft-water farm ponds in Georgia with no significant harm to largemouth bass. Applications of antimycin ranging from 0.4 to 0.8 ppb were most successful in ponds of 1 to 9 acres.

A typical operation involved a 2.5-acre bass-bluegill pond in which we needed to reduce the numbers of overabundant bluegills, golden shiners, and crappies with minimum damage to the largemouth bass. Only 0.4 ppb of antimycin applied to the 75^{o} F. water killed 127 pounds of golden shiners, 172 pounds of stunted bluegills, 22 pounds of fingerling crappies, and only 7 pounds of fingerling bass. No adult bass and crappies were killed. Moreover, subsequent reproduction by adult bass exposed to sublethal concentrations of toxicant was not impaired.

Antimycin's greatest potential as a selective toxicant possibly lies in the removal of scalefish from catfish waters. Minnows and sunfish, for example, infest many catfish production ponds in southeast and southcentral States, consuming large quantities of catfish rations, carrying diseases which affect the welfare of catfish, and necessitating costly sorting of scalefish and catfish at harvest.

Experiments at a catfish farm in Mississippi showed the benefits of reducing or eliminating abundant scalefish from production ponds. The ponds ranged from 0.9 to 1.4 acres, and averaged 120 pounds per acre of golden shiners and green sunfish. Ten ppb of antimycin killed at least 99 percent of the scalefish, despite high pH levels. No catfish perished. At harvest, treated ponds contained more and larger catfish than untreated ponds. The improved yield of catfish per acre averaged 330 pounds more in treated than in untreated ponds.

An excellent demonstration of selective poisoning was done by the Bureau's Division of Fishery Services on Victory Lake, Fort Benning, Ga. Elimination of scale fish from the 26-acre lake would permit effective management of channel catfish on a fed basis. Antimycin in liquid and sand formulations was used: 3.6 ppb in the lake proper and 10 ppb for brief periods in tributary streams and springs. This killed more than 5,200 pounds of scalefish, or 210 pounds per acre, comprising 60 pounds of chub suckers, 33 pounds of golden shiners, 55 pounds of bluegills and warmouth, and 19 pounds of largemouth bass. The small concentration had no effect on mosquitofish. Only one channel catfish and one bullhead were killed. Subsequent netting disclosed channel catfish up to 4.5 pounds and bullheads in good condition but no other species except mosquitofish. The job was a success, and intensive management of the catfish was begun soon after.

FORMULATIONS OF ANTIMYCIN

Handy formulations have been developed with antimycin without noxious or repelling carriers. The liquid formulation of antimycin is easily distributed in shallow waters and streams with spray apparatus or drip equipment. The dry formulations consist of antimycin coated on 40-mesh sand and designed for shallow ponds and littoral zones of lakes. Fintrol-5 is a preparation which releases the antimycin evenly within the first 5 feet of depth as the sand sinks to the bottom. It is handily distributed by seed spreaders mounted on boats or helicopters. Its advantage over liquid toxicant is that the granules do not stick to emergent or shoreline vegetation, but bounce off into the water. Moreover, the sand penetrates beds of submerged vegetation and better distributes the toxicant.

Fintrol-15 formulation is designed to release the toxicant into the water within the first 15 feet of depth. The selective removal of minnows from Pleasant Lake in Minnesota serves as an example of the usefulness of Fintrol-15 in deeper, thermally-stratified water. This lake, 80 feet deep, is managed for rainbow trout, but in recent years redbelly dace, bluntnose minnows, fathead minnows, blacknose shiners, and golden shiners had become abundant and heavily infested with parasites. The application was made to take advantage of the fact that the minnows prefer the upper layer of warm water in summer whereas the trout remain in the deep colder layers. When the lake was well stratified in late summer, the upper layer of water--the epilimnion--was approximately 15 feet thick. This layer was treated with Fintrol-15 and the shallower water along shore with Fintrol-5 to attain 5 ppb of antimycin in this upper stratum of warm water. As far as could be determined by trapping, netting, and SCUBA diving, the minnows were eliminated from the lake. There was a minor kill of rainbow trout which were presumed to have moved into the epilimnion to feed. Thus, treatment of the epilimnion of Pleasant Lake at the time of maximum thermal stratification was effective, practical, and much more economical than treating the entire body of water.

Fintrol-30 releases its antimycin within the first 30 feet of depth as the sand carrier sinks to the bottom. Its performance in penetrating to depths in thermally stratified water to reach target fish was observed in Beauty Lake in Quebec. The objective was to remove northern pike, minnows, white suckers, pumpkinseeds, and yellow perch to restore native brook trout in the 44-acre lake.

Beauty Lake has a maximum depth of 42 feet, and much of the lake exceeds 20 feet in depth--the shorelines are steep. Total treatment of the basin was necessary because the target species may have been distributed through a wide range of depths. We applied 5 ppb of antimycin as Fintrol-30 by boat to obtain as even a distribution as possible.

Fintrol-30 effectively released its antimycin in the deeper waters of the thermocline and was apparent within 2 hours after application. Large numbers of dead and dying American smelt rose to the surface of the lake. This species customarily inhabits the deeper, colder waters; indeed, its presence in Beauty Lake had never before been detected by fishery managers. Moreover, no live fish other than brown bullheads were taken during post-treatment netting in the lake. The operation was entirely successful in eradicating target fish.

Among these formulations, Fintrol-5 is registered and available to fishery managers. Fintrol-liquid, Fintrol-15, and Fintrol-30 may soon have registered labels. Other formulations may be developed, depending on the needs of fishery managers. A delayed-release formulation on sand--one which would release its toxicant only after the sand has sunk to the bottom-might be useful for control of lampreys, bullheads, and other bottom-dwelling fish.

DETOXIFICATION OF ANTIMYCIN

Laboratory and field tests confirmed that potassium permanganate can detoxify antimycin in water. The amount of permanganate depends on the concentration of toxicant, but the quantities, in general, are modest. However, we cannot recommend use of potassium where water is to be used for domestic or agricultural purposes or where fish are to be consumed by man because it has not been registered for this use by the U.S. Department of Agriculture.

The reclamation of Rathbone Creek at Cataract, Wis., required detoxification of antimycin. Several miles of the small, trout stream were treated with sufficient antimycin to maintain a concentration of 7.5 ppb for at least 8 hours in order to eliminate populations of minnows and suckers. Cataract Pond, a small impoundment, was the downstream limit of reclamation. It had been drained to low level before the treatment and refilled during treatment. Refilling only 18 hours before antimycin-laden water reached the spillway of the dam, the pond was treated with 2 ppm of potassium permanganate. The detoxification was quick and complete as shown by the fact that effluent water did not harm rainbow trout in live cages 500 feet downstream from Cataract Pond.

RECOMMENDATIONS FOR USE OF ANTIMYCIN OR OTHER FISH CONTROL AGENTS

The following considerations are essential to the successful reclamation of static or flowing waters.

I. Define the problem in full detail.

A. What is the cause of the problem?

B. What are the effects?

C. Is the problem temporary or long-term?

II. Assess the fish populations involved or present by netting, trapping, electro-fishing, or creel census.

III. Define the target species and their pertinent characteristics.

IV. Evaluate all possible approaches to a solution of the problem; include cost benefit ratios.

A. Biological or environmental controls.

B. Chemical controls:

1. General toxicant.
2. Selective toxicant.
3. Total reclamation.
4. Partial reclamation.

C. Other controls electrical, mechanical:

1. Electrical or mechanical weirs, barriers.
2. Dams, fishways.

D. Integrated controls--combinations of A, B, and C.

V. Select a control.

A. Consider its specificity to the target fish and life stages involved.

B. Consider possible side effects on other fish, aquatic life, and environment.

C. Consider effects or influences on multiple uses of the water.

If a chemical control is selected, the following additional considerations are advised. They apply to any of the registered fish toxicants. See table 5 for recommended concentrations.

I. Make several determinations of the following, preferably in both warm and cold seasons:

Item	Lake	Stream
A. General characteristics:		
1. Area	surface acres	surface acres.
2. Depth	av., contours	av., contours.
3. Volume	acre-feet	acre-feet.
4. Flows	current pattern	c.f.s.
5. Velocity	ft./sec.	ft./sec.

Item	Lake	Stream
6. Bottom:		
Littoral	type	type.
Profundal	type	type.
7. Tributaries	streams, springs	streams, springs.
8. Gradient	----	ft./mi.
9. Pool grade	----	type, frequency.
10. Riffle grade	----	type, frequency.
11. Vegetation	type, species	type, species.
12. Basin, channel	open, occluded	open, occluded.
13. Configuration	map, photograph	map, photograph.
B. Water characteristics:		
1. Chemistry	pH	pH.
Do	dissolved oxygen	dissolved oxygen.
Do	carbon dioxide	carbon dioxide.
Do	total alkalinity	total alkalinity.
Do	CO_3 alkalinity	CO_3 alkalinity.
Do	HCO_3 alkalinity	HCO_3 alkalinity.
Do	total hardness	total hardness.
2. Resistance	ohms	ohms.
conductance	mhos	mhos.
3. Temperature	$^oF.$, $^oC.$	$^oF.$, $^oC.$
4. Color	type	type.
5. Turbidity	type, density	type, density.
6. Pollution	type, source	type, source.

TABLE 5.--Guidelines for selecting concentrations of antimycin to control freshwater fish

	pH below 7.5		pH 7.5-8.5		pH above 8.5	
	Water Above 60°	Water Below 60°	Water Above 60°	Water Below 60°	Water Above 60°	Water Below 60°
Sensitive fish[1]:						
Antimycin A in ppb.........	5.0	7.5	7.5	10.0	10.0	10.0
Fintrol-5 in ppm...........	0.5	0.75	0.75	1.0	1.0	1.0
Fintrol-5 in pounds per acre foot................	1.4	2.0	2.0	2.8	2.8	2.8
Resistant fish[2]:						
Antimycin A in ppb.........	15	20	20	25	25	25
Fintrol-5 in ppm...........	1.5	2.0	2.0	2.5	2.5	2.5
Fintrol-5 in pounds per acre foot................	4.1	5.5	5.5	6.9	6.9	6.9

[1] Species such as gizzard shad, trout, pike, carp, minnows, suckers, sticklebacks, white bass, sunfish, perch, freshwater drum, and sculpins.
[2] Gar, bowfin, goldfish.

II. Correlate all data on target fish, on the lake or stream, and on water characteristics to aid in--

A. Selection of toxicant, formulation, and concentration for--

1. Total or partial reclamation.

2. General or selective control.

B. Selection of season and time for best application of control, with regard to--

1. Favorable temperature for chemical activity.

2. Favorable water chemistry for chemical activity.

3. Minimum conflicting uses of water.

4. Minimum interference by aquatic vegetation.

5. Limited vs. general distribution of target fish spawning congregations; preferred distribution in thermally-stratified water and life stages involved.

6. Favorable logistics to remote waters.

C. Selection of method for most effective distribution of toxicant (the distribution must be well planned, organized, and executed without interruption):

1. By hand--time, labor, costs.

2. By boat--time, labor, costs.

3. By aircraft--time, labor, costs.

D. Consider auxiliary tools to enhance effectiveness or economy of toxicant, e.g.--

1. Reduce interfering aquatic vegetation by prior application of herbicide.

2. Reduce turbidity with a flocculating material.

3. Manipulate water levels to concentrate fish, reduce water volume.

4. Modify the pH and chemistry of the water.

III. Confirm selection of toxicant, the formulation, and concentration for desired activity against target fish by lakeside or streamside bioassays, as follows:

A. Several concentrations of toxicant and durations of exposure which bracket the proposed application rate must be tested in the bioassays.

B. The water in the bioassays must be from the target lake or stream.

C. Temperatures in the bioassays must be representative of the target lake or stream.

D. Target species from the target lake or stream are preferred in the bioassays.

IV. Adjust the type, formulation, and concentration of toxicant and the time of reclamation as indicated by results of the bioassays.

V. Set live cages containing target and other indicator species at various sites and depths in the target water at least 24 hours prior to the reclamation. Check their survival just before the reclamation and periodically thereafter. Replace dead fish with fresh lots of specimens to detect as accurately as possible the persistence and activity of the toxicant. A lot of fish must include at least 10 specimens of the same species and size. When a fresh lot of the most sensitive species survives 48 hours of exposure in the treated water, the toxicant is considered degraded or removed from the basin.

VI. Postpone or cancel the reclamation if any conditions in the target water or reclamation process have changed significantly since the bioassays were made. The risk of failure--always present--becomes too high when conditions have changed.

VII. Collect, enumerate, process, and bury dead fish promptly.

VIII. Make thorough assessment of the results of the reclamation, by--

A. Netting, trapping, electrofishing, or SCUBA.

B. Detect and evaluate side effects on any aquatic or terrestrial life.

IX. Relate results to objectives. Were the objectives attained?

X. Clean up the reclamation site; properly dispose of surplus toxicant and empty containers.

XI. Prepare full report of the reclamation for files and/or publication.

XII. Prepare and distribute an addendum report on the type and success of post-reclamation management of the fishery.

REFERENCES

Ayerst Laboratories.
1966. Fintrol (R). A new chemical concept for the eradication of rough and stunted freshwater fish. Ayerst Laboratories, New York, N.Y. 10 p.

Ayerst Laboratories.
1967. Fintrol in catfish farming eliminates trash fish. Ayerst Laboratories, New York, N.Y. 11 p.

Barry, James J. and Richard E. Bass.
1967. Trial application of Antimycin A in three hatchery ponds. Department of Natural Resources, Avoca, Ind. 5 p. [Mimeo].

Berger, Bernard L.
1965. An application of Fintrol-5 (antimycin) to Veterans Memorial Park Pond, West Salem, Wisconsin. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., November 12: typed. 11 p.
1965. A trial of antimycin A in ponds on the Valentine National Wildlife Refuge. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., June 8: typed. 6 p.
1966. Field trials of Fintrol-5 at Stuttgart, Arkansas, in December 1965. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., March 7: typed. 20 p.
1966. Antimycin (Fintrol) as a fish toxicant. Proceedings of the Nineteenth Conference of the Southeastern Game and Fish Commissioners, Tulsa, Oklahoma, October 10-13. 1965. p. 300-301.
1966. Reclamation of Lake Creek Lake with Fintrol-5 at the National Fish Hatchery, Saratoga, Wyoming. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., January 13: typed. 13 p.

Berger, Bernard L., Philip A. Gilderhus, and Robert E. Lennon
1967. Attempted reclamation of Whitewater Lake, Valentine National Wildlife Refuge, Nebraska. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., October 25: mimeo, 12 p.

Berger, Bernard L., and Robert E. Lennon.
________. A test of Antimycin A, a fish toxicant, in Beauty Lake Quebec, Canada. Canadian Fish Culturist. (In press).

Berger, Bernard L., Robert E. Lennon, and James W. Hogan.
1969. Laboratory studies on antimycin A as a fish toxicant. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 26. 19 p.

Burress, Ralph M.
1968. Antimycin for controlling sunfish populations in ponds. Farm Pond Harvest, vol. 1, No. 2, p. 11-12-22.

Burress, Ralph M., and Charles W. Luhning.
1969. Use of antimycin for selective thinning of sunfish populations in ponds. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 28. 10 p.
1969. Field trials of antimycin as a selective toxicant in channel catfish ponds. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 25. 12 p.

Derse, P. H., and F. M. Strong.
1963. Toxicity of antimycin to fish. Nature, vol. 200, No. 4906, p. 600-601.

Gilderhus, Philip A.
1967. Field studies with antimycin in Waterloo Creek at Marshall, Wisconsin. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., August 16: typed, 3 p.
1966. Summary of the reclamation of Rathbone Creek with antimycin. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., November: typed, 7 p.
1967. The reclamation of Westfield Creek, Marquette County, Wisconsin, using antimycin. Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., August 30: typed, 7 p.

Helms, Don R.
1967. Experimental use of antimycin A to control spawning carp in Backbone Lake. Iowa Conservation Commission: typed, 5 p.

Herr, F., E. Greselin, and C. Chappel.
1967. Toxicology studies of antimycin, a fish eradicant. Transactions of the American Fisheries Society, vol. 96, No. 3, p. 320-326.

Heylin, Mike.
1965. Antimycin kills freshwater fish in parts-per-billion dosages. Chemical and Engineering News, vol. 43, No. 44, p. 52.

Hogan, James W.
________. Antimycin as a fish toxicant in catfish culture. Proceedings of the Twentieth Conference of the Southeastern Association of Game and Fish Commissioners, Asheville, North Carolina, October 24-26, 1966: (In press).

Iowa. State Conservation Commission.
1967. Rough fish and species control Fish Management Quarterly Report, vol. 1, No. 2, p. 21-29.

Lennon, Robert E.
1966. Antimycin as a fish toxicant. Presented at 96th Annual Meeting, American Fisheries Society, Kansas City, Mo., September 12-14: mimeo (Fish Control Laboratory, La Crosse, Wis.), 8 p.
1966. Antimycin - a new fishery tool. Wisconsin Conservation Bulletin, vol. 31, No. 2, p. 4-5.

Lennon, Robert E., Bernard L. Berger and Philip A. Gilderhus.
1967. A powered spreader for antimycin. Progressive Fish-Culturist, vol. 29, No. 2, p. 110-113.

Loeb, H. A.
1964. Some notes concerning the toxicity of Antimycin A to carp and other fish. New York Fish and Game Journal, vol. 11, p. 160-161.

Montgomery, Alex B.
1967. Fishery management program, Shaw Air Force Base, South Carolina. Summary Report, Division of Fishery Services, U.S. Bureau of Sport Fisheries and Wildlife, October 17: typed, 3 p.
1967. Fishery management program, Fort Benning, Georgia. Progressive Report, Division of Fishery Services, U.S. Bureau of Sport Fisheries and Wildlife, October 23: typed, 3 p.

Powers, James E., and Anne LaBastille Bowers.
1967. Elimination of fish in the Giant Grebe Refuge, Lake Atitlan, Guatemala, using the fish toxicant, Antimycin. Transactions of the American Fisheries Society, vol. 96, No. 2, p. 210-213.

Powers, James E., and Edward Schneberger.
1967. Antimycin: Promising in carp control. Wisconsin Conservation Bulletin, vol. 32, No. 2, p. 14.

Radonski, Gilbert C.
1967. Antimycin: Useful in perch control? Wisconsin Conservation Bulletin, fol. 32, No. 2, p. 15-16.

Ross, Ward.
1966. Carp control research contract. Quarterly Progress Reports Nos. 1-3.

Ross, Ward.
1967. Carp control research contract. Quarterly Progress Reports, Nos. 4-5. Wisconsin Alumni Research Foundation, Madison, Wis. mimeos.

Sayre, R. C.
1967. Powder River rehabilitation with Fintrol-5 and liquid rotenone. Progress Report No. 2, Project F-80-R-1, Oregon State Game Commission, December 13: mimeo, 12 p., 17 figs.
1967. Fintrol-5 for fish eradication. Powder River rehabilitation. Progress Report, Project F-80-R-1, Oregon State Game Commission, November 10: mimeo, 5 p., 5 figs.

Schoettger, Richard A., Bernard L. Berger, and Philip A. Gilderhus.
1967. Reclamation of Turquoise Lake, Leadville, Colorado. Trip Report, Fish Control Laboratory; U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wisconsin, September: mimeo, 20 p.

Sheridan, John R.
1967. Fishery management program, Fort Detrick, Maryland. Progress Report, Division of Fishery Services, U.S. Bureau of Sport Fisheries and Wildlife, October 26: typed, 5 p.

Vezina, Claude.
1966. Antimycin A, a teleocidal antibiotic. Antimicrobial Agents and Chemotherapy--1966, p. 757-766.

Walker, Charles R., Robert E. Lennon, and Bernard L. Berger.
1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 2. (Bureau Circular 186). 18 p.

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