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INVESTIGATIONS IN FISH CONTROL

Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals

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Abstract.--Antimycin A, an antifungal antibiotic, has been suggested for use as a fish toxicant. Preliminary tests were made to evaluate its effects at concentrations of 0.01 to 120 p.p.b. on 24 species of fresh-water fish in the laboratory and 25 species in outdoor pools. Responses of a select group of other animals and aquatic plants are discussed. The antibiotic is a powerful fish toxicant. Carp and other rough fish were killed by small concentrations in short exposures at cool and warm temperatures. Longnose gar, bowfin, black bullheads, and yellow bullheads were relatively resistant to the quantities tested. Plankton, aquatic plants, bottom fauna, salamanders, tadpoles, and turtles were not harmed by pisicicidal concentrations. Antimycin A degrades rapidly in water, especially in the presence of free hydroxide. Detoxification occurred within 24 to 96 hours. Further studies are planned on the performance of antimycin A against various life stages of fish, on other aquatic animals, and in waters of differing qualities and temperatures. The process of detoxification and the fate of residues deserve further attention.

An objective of the Fish Control Laboratories is the development of new fish toxicants that can be used safely and economically in the management of fish populations. Antimycin A exhibits properties desired in a candidate fish toxicant. It is lethal to certain target fishes in low concentration and on short exposure; it works in cool and warm water and in the presence of aquatic plants; it degrades rapidly in water and appears to leave no harmful residue.

This report summarizes data obtained on antimycin A in the laboratory and small outdoor pools and larger hatchery ponds. Development and efficacy of the compound as a fishery tool is to be further investigated.

ANTIMYCIN

Sources and uses

Antimycin is an antifungal antibiotic isolated from the bacteria *Streptomyces* sp. and identified by Dunshee, Leben, Keitt, and Strong (1949) at the University of Wisconsin. Following this discovery, at least seven species of *Streptomyces* were found to be producers of antimycin. Burger, Teitel, and Grunberg crystalized the antibiotic from two species of *Streptomyces* (Strong, 1956). Later at the University of Wisconsin, another culture produced an antimycin-like product which showed promise as an antibiotic for plant pathogens (Lockwood et al., 1954).

Harada and associates (Nakayama et al., 1956) in Japan discovered an antimycin-producing culture of *Streptomyces kitazawaensis* which differed from the first culture at the University of Wisconsin, but both produce an antitumor substance *(carzinomyceticus).* Research at the University of Tokyo by Watanebe et al. (1957) on *S. blastmyceticus* yielded an antibiotic called blastmycin which consists largely of antimycin A3. Harada et al. (1959) devoted special attention to the antifungal property of blastmycin as a control for rice blast disease *(Piricularia oryzae)* in Japan.

Derse and Strong (1963) related that antimycin is an antibiotic of unusual chemical structure which is toxic to yeasts, other fungi, insects, and mammals, but not to bacteria. They also reported that it is extremely toxic to goldfish at 1 p.p.b. On the basis of this observation, on the rapid degradation of the chemical, and its much lower toxicity to higher animals, they suggested that antimycin may be useful in fish management.

Composition and structure

The complex structure of antimycin was elucidated by Dunshee et al. (1949), Tener et al. (1953), Strong (1956) and Strong et al. (1960), van Tamelen et al. (1959 and 1961), and Dickie et al. (1963). It is illustrated in figure 1.

Lockwood et al. (1954) described antimycin as a complex made up of several active fractions which they identified from paper chromatograms as A1, A2, A3, and A4 according to increasing R_F values. Liu and Strong (1959) determined that one or more of these R_F values were represented in antimycin A-35, antimycin A-102, blastmycin, and virosin, and they investigated them. Further study by Dickie and his associates (1963) established that the fractions differ only in the alkyl side chain (R) in figure 1. The antimycin A1 and A4 fractions are probably isomeric with R = n-hexyl, and calculations of the elemental composition indicate that the emperical formula is $C_{28}H_{40}N_2O_9$. The A2 and A_3 isomers bear the n-butyl side chain, and the emperical formula is perhaps $C_{26}H_{36}N_2O_9$. The percentage composition of fractions or isomers is very important to the biological activity of the antimycin complex.

Physical and chemical properties

The fermentation extracts of antimycin are dark, tarry substances which upon further purification yield a fine crystalline material. This nitrogenous, phenolic complex is characterized by solubility in polar organic solvents including ethanol, acetone, and chloroform; slight solubility in nonpolar solvents including petroleum ether, benzene, and carbon tetrachloride; and relative insolubility in water and 5-percent solutions of hydrochloric acid, sodium bicarbonate, and sodium carbonate (Keitt, Leben, and Strong, 1953).

The infrared absorption spectrum of antimycin has been identified in isolates from several cultures, although the crystalline products appear to have different properties. These differences are attributed to the intricate composition of the antibiotic and the presence of impurities associated with samples (Strong, 1956). For example, blastmycin has almost the duplicate IR spectrum of antimycin A-35 isolate, but the melting points are 166°-167° and 140.5°-141.5° C. respectively. Blastmycin is composed primarily of the antimycin A_3 fraction with a trace of A4 in contrast to antimycin A-35, antimycin A-102, and virosin, which contain additional subcompounds A1 and A_2 (Strong, 1956; Liu and Strong, 1959).

Antimycin is susceptible to alkaline degradation as indicated in figure 1. Hydrolytic cleavage occurs at the lactone carbonyl sites on the cyclic diester and leads to the formation of antimycic acid or blastmycic and the neutral fragment (van Tamelen et al., 1961; Liu et al., 1960; and Tener et al., 1953). The degradation is rapid in water, and detoxification of 10 p.p.b. is accomplished within 7 days according to Derse and Strong (1963); it is accelerated in the presence of light, high alkalinity, and warm temperatures.

Biological activity

Antimycin is a powerful and highly selective inhibitor of the electron transport in oxidative

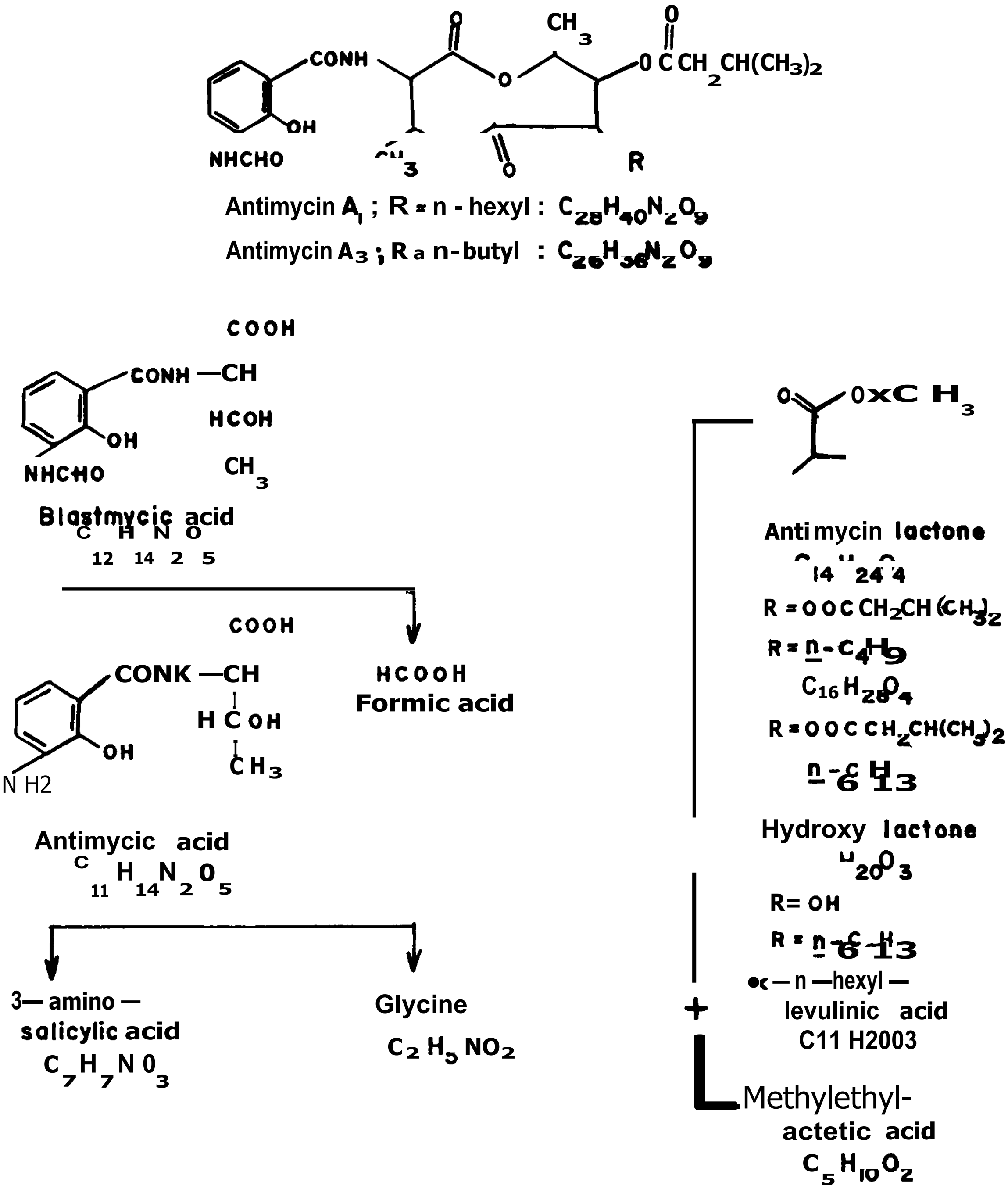


Figure 1.--Structure of antimycin and the assumed process of breakdown under alkaline conditions in the laboratory.

phosphorylation systems (Strong, 1956). It retards the respiration of cells, and the selective action-in the electron transport chain at the cytochrome b - (Coenzyme Q)-cytochrome c has made antimycin an indispensable reagent for enzyme studies. Its effects on the succinic-oxidase system have been described as the "antimycin-A-blocked factor." Gottlieb and Ramachandran (1961) illustrated the site of action of antimycin and ascosin as follows:

Substrate --> Pyridine nucleotide--> Flavoprotein-->

- - -> < ,cytochrome b / 'coenzyme - "AS" site -->

cytochrome c--> cytochrome a--> oxygen

Because of its extreme potency as an inhibitor of electron transport, Derse and Strong (1963) surmised that antimycin is absorbed into the gills and interferes with respiration in fishes.

METHODS AND MATERIALS

Crystalline antimycin A was supplied by the Wisconsin Alumni Research Foundation from Kyowa Fermentation Company, Ltd., in Tokyo, Japan. This material was isolated from the culture of *Streptomyces kitazawaensis* and had the following fractions by weight: A_1, 40 percent; A2, 20 percent; A3, 20 percent; and A4, 10 percent. Although the fraction A3 amounts to only 20 percent, it accounts for about 60 percent of the biological activity.

Stock solutions were prepared with 100 milligrams of crystalline antimycin A dissolved in 1 liter of acetone. They were renewed with each series of bioassays, although tests indicated that solutions in acetone are relatively stable up to 24 days. Crystalline material stored at room temperature for 2 years also remained stable.

Laboratory tests

The methods and facilities employed for evaluation of potential fish-control agents were described by Lennon and Walker (1964). The bioassays of antimycin A were conducted in slightly alkaline and medium hard, reconstituted water at 12°, 17°, and 22° C. Twenty-four species of fish, representing nine families, were included (table 1). They were supplied by national fish hatcheries, the Wisconsin Conservation Department, and Ozark Fisheries, Inc., and each lot was graded to a desired size before use.

Aliquots of the stock solution of antimycin A were diluted and stirred into the 1- or 5-gallon bioassay vessels in the presence of fish. The responses of the fish to the toxicant were observed at 24, 48, 72, and 96 hours.

Other animals included in bioassays were water fleas *(Daphnia magna),* crayfish *(Cambarus* sp.), damselfly nymphs *(Ischnura* sp.), tiger salamander *(Ambystoma tigrinum),* and bullfrog tadpoles *(Rana catesbiana).* They were stocked in bioassay vessels as follow: 10 water fleas or 2 damselfly nymphs in each 16-ounce jar, 1 crayfish or 2 bullfrog tadpoles in each 1-gallon jar, and 1 adult tiger salamander in each 5-gallon jar.

Field tests

Vinyl wading pools.--Only a few outdoor bioassays were made in 1962 and 1963 because only small quantities of toxicant were available.

TABLE 1.--The 24 fishes used in laboratory tests of antimycin A

Common name	Technical name	Size range (grams)
Gizzard shad	Dorosoma cepedianum	12.0-15.0
Rainbow trout	Salmo gairdneri	1.0- 1.6
Brown trout	Salmo trutta	1.2- 1.4
Northern pike	Esox lucius	0.5- 0.6
Stoneroller	Campostoma anomalum	3.0- 4.0
Goldfish	Carassius auratus	1.5- 2.4
Carp	Cyprinus carpio	0.6- 2.3
Golden shiner	Notemigonus crysoleucas	1.0- 2.2
Fathead minnow	Pimephales promelas	0.9- 1.8
White sucker	Catostomus commersoni	1.3- 2.8
Bigmouth buffalo	Ictiobus cyprinellus	1.6- 2.5
Black bullhead	Ictalurus melas	0.7- 2.3
Yellow bullhead	Ictalurus natalis	1.2- 2.5
Channel catfish	Ictalurus punctatus	1.5-,1.8
Brook stickleback	Eucalia inconstans	0.6- 1.0
Green sunfish	Lepomis cyanellus	0.8- 2.5
Pumpkinseed	Lepomis gibbosus	1.0- 2.3
Bluegill	Lepomis macrochirus	1.2- 2.4
Longear sunfish	Lepomis megalotis	1.0- 2.5
Largemouth bass	Micropterus salmoides	1.8- 2.9
White crappie	Pomoxis annularis	1.5- 3.0
Iowa darter	Etheostoma exile	0.6- 1.2
Yellow perch	Perca flavescens	0.6- 3.0
Walleye	Stizostedion vitreum	0.4- 0.8

The test vessels were 1,000-gallon wading pools similar to those described by Lawrence and Blackburn (1962). Some physical, chemical, and biological conditions characteristic of ponds were simulated or intrinsic. The physical aspects included bottom soils of sand and loam, naturally varying temperatures, turbidity, and natural light. The chemistry of the well water in the pools was modified by physical and biological factors.

Of the 18 pools, 9 had 3 inches of sand on the bottom, and 9 had 3 inches of silt loam. After the pools were filled, the following were introduced: *Sagittaria latifolia, Elodea canadensis, Myriophyllum heterophyllum, Potamogeton nodosus, P. pectinatus, Spirogyra* spp., and phytoplankton. They were established, and the water chemistry was stabilized, during the 4- to 8-week periods before fish were added. Fingerling and adult fish were stocked 1 to 2 weeks before applications of the toxicant.

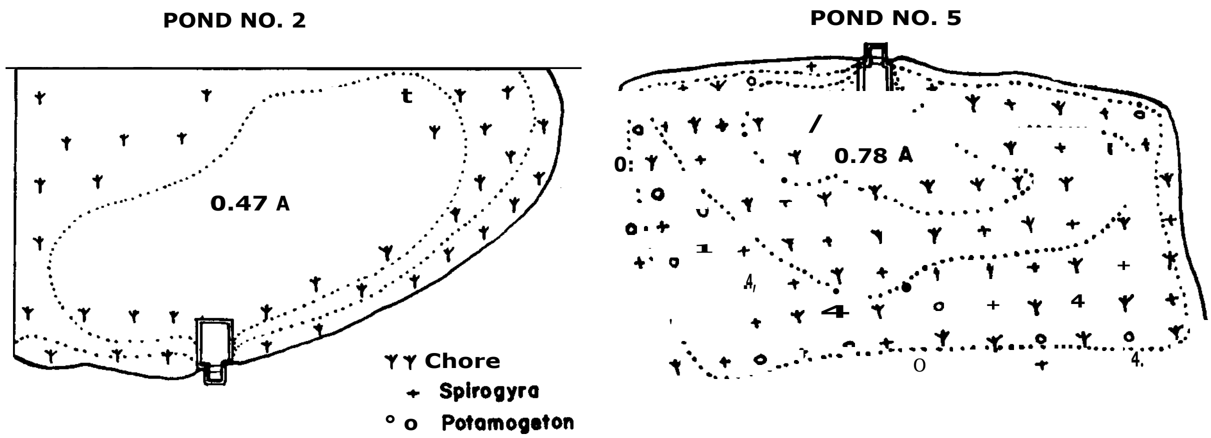
The rate of detoxification of the antimycin was observed, and some of the killed fish were shipped to the Wisconsin Alumni Research Foundation for mammalian toxicity tests. Bottom fauna were sampled and quantitated. Data were obtained on water chemistry during the course of tests according to standard methods (American Public Health Association et al., 1960).

Hatchery ponds.--The Wisconsin Conservation Department provided two ponds for tests at the Delafield Warmwater Fisheries Research Station in September 1963. The surface areas of ponds No. 2 and No. 5 are 0.47 and 0.78 acre respectively (fig. 2).

Pond No. 2 was stocked with 18 species of fish at the rate of 240 pounds per acre, and pond No. 5 with 19 species at 225 pounds per acre, 1 week before antimycin was applied. Samples of water, plankton, and bottom fauna were taken from each pond soon after the fish were stocked and again just before the ponds were drained (table 2).

TABLE 2.--Concentrations of antimycin A which caused all-or-none survival among rainbow trout and brown trout at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (° C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Rainbow trout..	1,829	12	0.10	0.60	0.02	0.08
Do........	120	17	0.02	0.08	<0.02	0.04
Brown trout....	348	12	0.10	0.40	<0.06	0.08
Do........	120	17	0.02	0.06	<0.04	0.06



CONTOUR	ACRE-FEET
0 - 1 ft.	0.44
1 - 2ft.	0.33
2 + ft.	0.01
TOTAL	0.78

CONTOUR	ACRE-FEET
0 - 1 ft.	0.68
1 - 2 ft.	0.63
2 - 3 ft.	0.42
3+ ft.	0.17
TOTAL	.90

Figure 2.--Sketch of ponds No. 2 and No. 5 at the Delafield Warmwater Fisheries Research Station.

Two formulations of antimycin A were prepared for application at 10 p.p.b. Pond No. 2 received 9.72 grams of technical material in a carrier formulated by the S. B. Penick Company to make up a total volume of 300 ml. Pond No. 5 received 23.37 grams of technical material dissolved in 300 ml. of acetone as a carrier. Each aliquot was mixed with 2 gallons of water and applied to a pond surface with a hand-powered garden sprayer. The applications were made from a rowboat in late afternoon, and frequent observations were made during the next 8 hours. Observations and recovery of dead fish were made daily in the following 4 days.

RESULTS OF LABORATORY STUDIES

We found that antimycin A is toxic to the 24 species of fish tested. The toxicity varies among species and is correlated with water temperature and time. Trends in sensitivity reflect taxonomic relationships of the fishes, and variations in susceptibility among individuals was more pronounced in some species than others. The following remarks pertain principally to the concentrations which delineate the all-or-none survival EC0 to EC100 ranges, of fish at 24 or 96 hours in bioassays at 12°, 17°, or 22° C. Data are shown graphically in figures 3 and 4.

Among the 24 species, the group of fish most sensitive to antimycin A includes gizzard shad, rainbow trout, brown trout, white sucker, Iowa darter, yellow perch, and walleye. All survived exposure to 0.08 p.p.b. for 24 hours at 12° C; all perished at 0.8 p.p.b.

The group intermediate in sensitivity included northern pike, stoneroller, carp, golden shiner, fathead minnow, bigmouth buffalo, brook stickleback, green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie (fig. 5). Concentrations of 0.1 and 1.6 p.p.b. defined their all-or-none survival in 24 hours at 12° C.

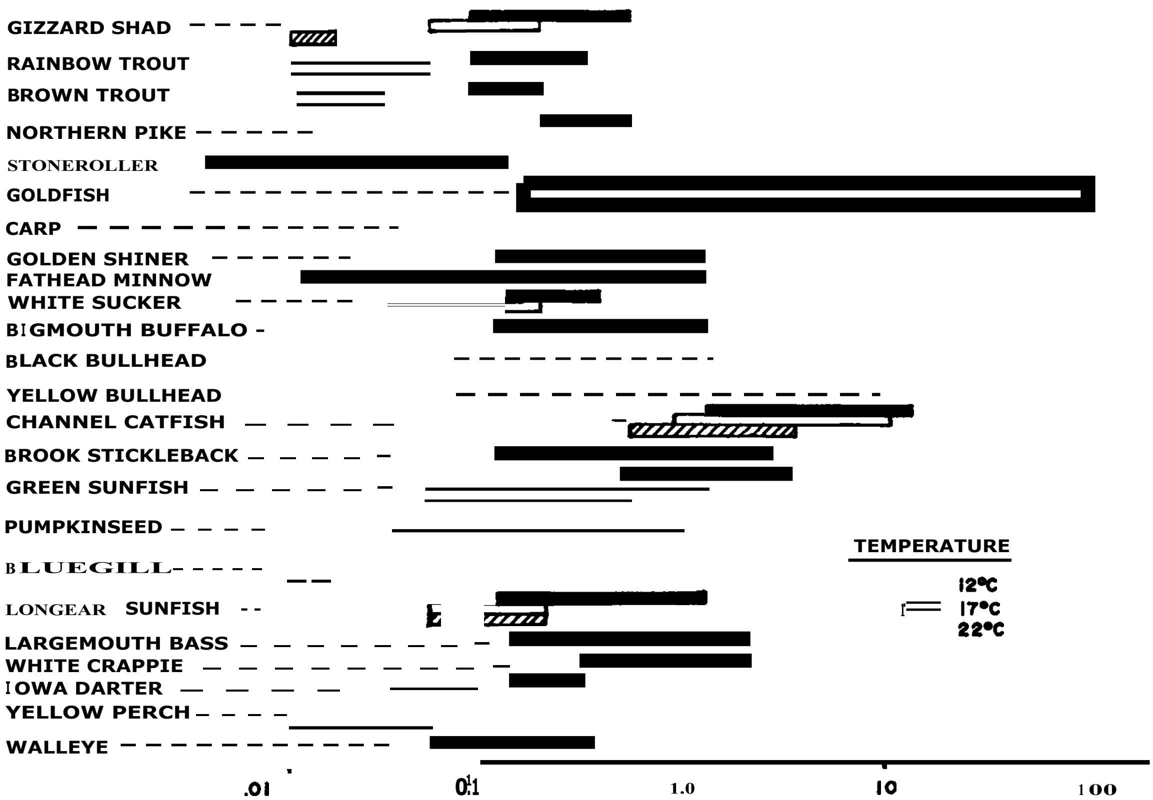


Figure 3.--The 24-hour responses of 24 fishes in the laboratory to antimycin A in p.p.b. The solid, plain, and cross hatched bars span the ranges between the EC_0 and EC100 at 12°, 17°, and 22° C.

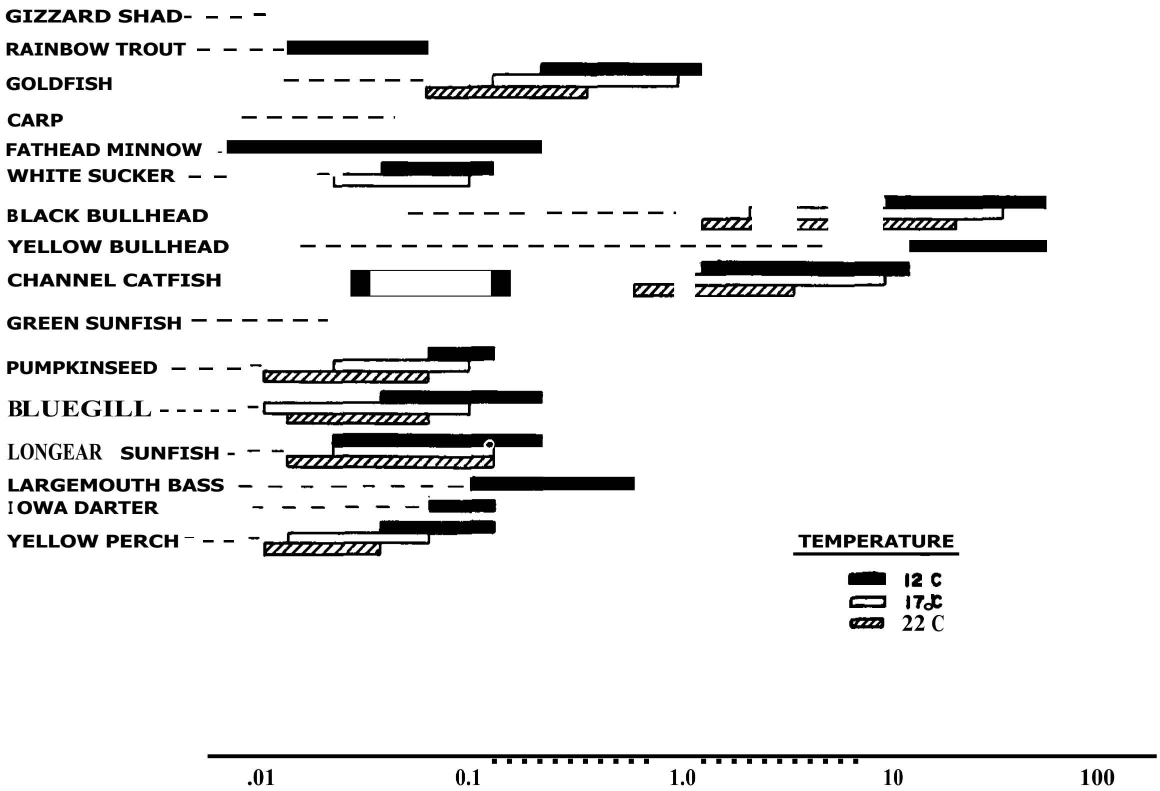


Figure 4.--The 96-hour responses of 16 fishes in the laboratory to antimycin A in p.p.b, The solid, plain, and crosshatched bars span the ranges between the EC_0 and EC_{100} at 12°, 17°, and 22° C.

The more resistant group of fish was represented by goldfish, black bullhead, yellow bullhead, and channel catfish. The concentrations required for kills in 24 hours at 12° C were 20 p.p.b. for channel catfish, 80 p.p.b. for yellow bullhead, 100 p.p.b. for goldfish, and 120 p.p.b. for black bullhead.

Increases in water temperature or duration of exposure made significant differences in the toxicity of antimycin A to fish in the three groups. For example, the toxicity to goldfish was increased tenfold at the higher temperature of 17° C. Among catfishes, the toxicity was enhanced about twofold at 17°. At the maximum temperature of 22°, the black and yellow bullheads were about 10 times as tolerant to antimycin A as goldfish, but channel catfish were only slightly more resistant.

For more detailed discussion on the toxicity of antimycin A, the species are grouped according to their respective families. The families, in turn, are presented in order of their sensitivity to the toxicant.

Trouts

Rainbow trout and brown trout were extremely sensitive to antimycin A (table 2). At 12°, the rainbow trout succumbed to 0.6 p.p.b. in 24 hours and to 0.08 p.p.b. in 96 hours. At the same temperature, brown trout were killed by 0.4 p.p.b. in 24 hours and by 0.08 p.p.b. in 96 hours. Both species tolerated concentrations of 0.1 p.p.b. for 24 hours. In 96-hour tests, the rainbow trout survived 0.02 p.p.b. whereas brown trout withstood 0.06 p.p.b.

Herrings

At 12° C., all gizzard shad died within 24 hours upon exposure to 0.8 p.p.b. and within 96 hours at 0.1 p.p.b. (table 3). They were especially sensitive to the toxicant at 22°:

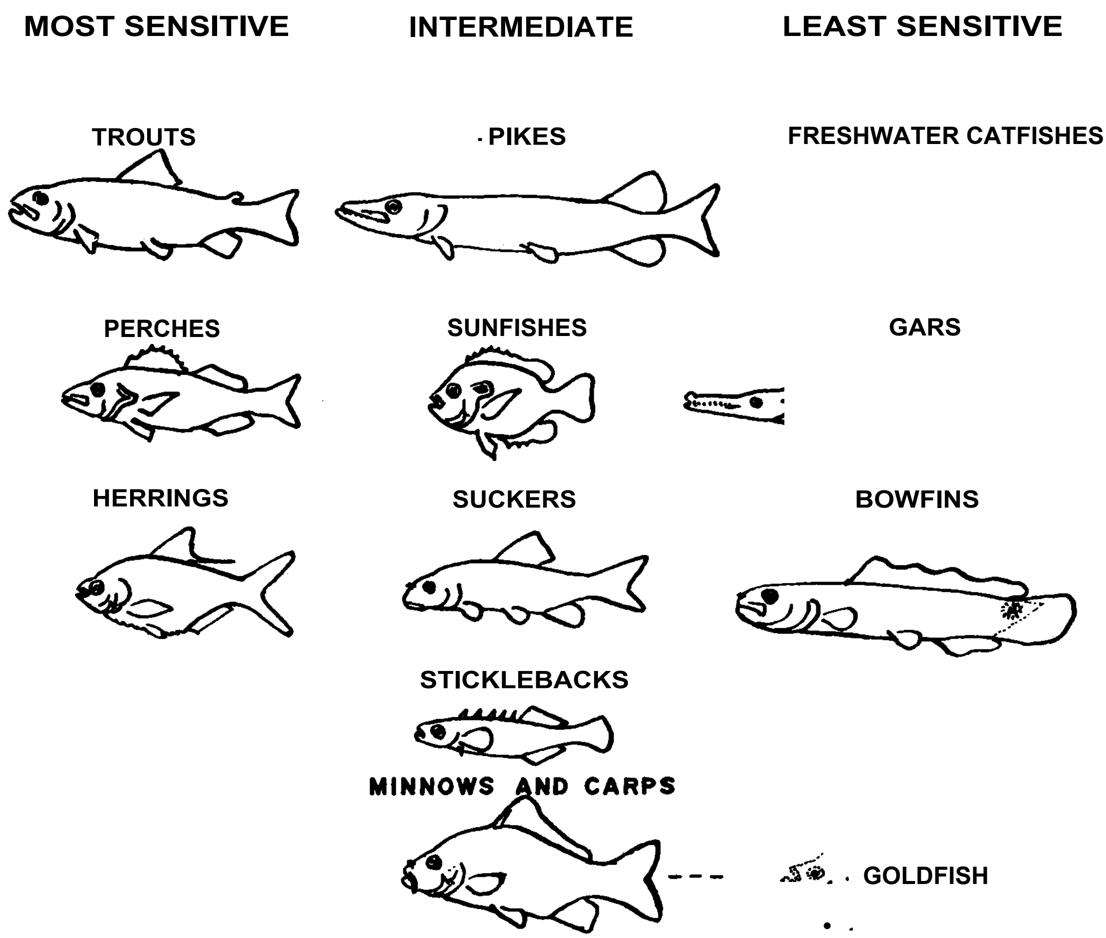


Figure 5.--The order of sensitivity of 11 families of fish to antimycin A in the laboratory and field.

concentrations of 0.04 p.p.b. caused complete kills within 24 hours, and partial kills occured at 0.02 p.p.b. or more. It was noted that a narrow range of concentrations yielded all-or-none survival, particularly at the higher temperature and longer exposure.

Perches

The Iowa darter, yellow perch, and walleye were also very sensitive to antimycin A (table 4). All specimens in 0.08 p.p.b. at 12° for 24 hours survived, but those in 0.66 p.p.b. died. The narrow range in concentrations which caused all-or-none survival was more apparent at 22° and 96-hour exposures. Yellow perch, for example, survived 0.02 p.p.b. for 24 hours and 0.01 p.p.b. for 96 hours; they died at 0.08 p.p.b. within 24 hours and at 0.06 p.p.b. within 96 hours.

Pikes

The fry and fingerlings of northern pike were difficult to use in bioassays because of cannibalism and rapid growth. Nevertheless, they exhibited great susceptibility to antimycin A. Complete kills were obtained in 24

TABLE 3.--Concentrations of antimycin A which caused all-or-none survival among gizzard shad at selected water temperatures in 24 and 96 hours

Number of fish	Temperature (C)	Concentrations (p.p.b.) and survival			
		At 24 hours		At 96 hours	
		All	None	All	None
120	12	0.10	0.80	0.06	0.10
60	17	0.08	0.40	0.04	0.08
60	22	0.02	0.04	0.02	0.04

hours by 0.8 p.p.b. at 12°, 0.2 p.p.b. at 17°, and 0.1 p.p.b. at 22°. In contrast, all specimens survived 0.4 p.p.b. at 12°, 0.08 p.p.b. at 17°, and 0.06 p.p.b. at 22°. Greater toxicity was detected in 48-hour exposures, but the concentrations related to all-or-none survival were not defined.

Suckers

The white sucker and bigmouth buffalo differed in their sensitivities to the toxicant, and the former was among the most susceptible fishes tested (table 5). Concentrations greater than 0.06 p.p.b. produced partial kills of white suckers at 12° in 96 hours, and 0.22 p.p.b. caused complete kills. Even greater sensitivity was observed at 22°. The bigmouth buffalo, on the other hand, required concentrations of antimycin A in excess of 0.4 p.p.b. for complete kills in 96 hours at 12°.

Sunfishes

Green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie were moderately sensitive to antimycin A (table 6). The concentrations required to cause complete kills of them at 12° ranged from 1 to 6 p.p.b. in 24 hours and from 0.2 to 0.8 p.p.b. in 96 hours. At 22°, killing concentrations ranged from 0.2 to 0.8 p.p.b. in 24 hours and from 0.08 to 0.4 p.p.b. in 96 hours.

The pumpkinseed and bluegill were the more sensitive of the six species, and they were followed in order of decreasing sensitivity by longear sunfish, largemouth bass, white crappie, and green sunfish.

Sticklebacks

Brook sticklebacks were moderately sensitive to antimycin A at 12°. Concentrations of 5 p.p.b. killed all specimens within 24 hours, and partial kills occurred at concentrations greater than 0.5 p.p.b. The exposures beyond 24 hours failed to give consistent results. The condition of the fish was suspect because of difficulty in maintaining them without feeding during the longer test periods.

Minnows and carps

Stoneroller, goldfish, carp, golden shiner, and fathead minnow responded over a wide range of concentrations in an interesting pattern of susceptibility. In contrast to other families, the minnows exhibited greater variation in response between species as well as between individual speciments (table 7).

TABLE 4.--Concentrations of antimycin A which caused all-or-none survival among Iowa daters, yellow perch, and walleye at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (°C.)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Iowa darters..	275	12	0.10	0.66	0.08	0.14
Yellow perch..	560	12	0.10	0.40	0.06	0.20
Do.......	504	17	0.08	0.40	0.02	0.08
Do.......	60	22	0.02	0.08	0.01	0.06
Walleye.......	20	12	0.08	0.60	--	--
Do.......	20	17	<0.08	0.10	--	--

TABLE 5.--Concentrations of antimycin A which caused all-or-none survival among white sucker and bigmouth buffalo at selected temperatures in 24 and 96 hours

Species	Number of fish	Temperature (°C.)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
White sucker..	810	12	0.22	0.64	0.06	0.22
Do.......	36	17	0.06	0.40	0.04	0.10
Do.......	72	22	<0.04	0.20	<0.06	0.10
Bigmouth buffalo	430	12	0.20	2.00	<0.10	0.40

TABLE 6.--Concentrations of antimycin A which caused all-or-none survival among green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (°C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Green sunfish..	396	12	0.80	6.00	0.04	0.80
Do........	216	17	0.20	2.00	0.08	0.60
Do........	30	22	0.08	0.80	0.08	0.40
Pumpkinseed....	480	12	0.40	2.00	0.08	0.20
Do........	120	17	0.06	1.00	0.04	0.10
Do........	180	22	0.01	0.20	0.01	0.08
Bluegill......	1,053	12	0.20	1.00	0.06	0.40
Do........	360	17	0.10	0.60	0.01	0.10
Do........	200	22	0.04	0.20	0.02	0.08
Longear sunfish.	240	12	0.20	2.00	0.04	0.40
Do........	48	17	0.08	0.40	0.04	0.20
Do........	48	22	0.08	0.40	0.02	0.20
Largemouth bass.	800	12	0.20	<6.00	0.10	0.80
White crappie..	180	12	0.60	>2.00	--	--

TABLE 7.--Concentrations of antimycin A which caused all-or-none survival among stoneroller, goldfish, carp, golden shiner, and fathead minnow at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (° C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Stoneroller....	531	12	0.08	∞	--	--
Goldfish......	1,469	12	4.00	00.∞	0.40	2.00
Do........	312	17	1.00	8.∞	0.20	1.00
Do........	200	22	0.30	4.∞	0.08	0.60
Carp..........	240	12	0.60	∞	0.08	0.60
Do........	84	17	0.40	∞	0.08	0.40
Golden shiner..	60	12	0.20	2.∞	0.05	0.60
Do........	60	17	0.10	0.5	--	--
Do........	40	22	0.05	∞	--	--
Fathead minnow..	816	12	0.10	2.∞	0.08	0.40
Do........	96	17	<0.80	2.∞	<0.06	0.10
Do........	78	22	<0.10	0.∞	<0.10	0.10

An outstanding highlight of the screening program was the discovery that carp are vulnerable to small concentrations of antimycin A. This prolific exotic is widely con-. sidered a most undesirable species in gamefish waters and is difficult to control with existing means.

At 12° all test carp were killed by 2 p.p.b. of antimycin in 24 hours and by 0.6 p.p.b. in 96 hours; at 17° all were killed by 1 **p.p.b.** in 24 hours and by 0.4 p.p.b. in 96 hours. Temperatures had less effect on toxicity to carp than to most species. There were only slight differences due to temperature in 24-hour exposures and even less at 96 hours. All carp survived 0.08 p.p.b.

The results on goldfish contrasted sharply with those on carp. In fact, the goldfish was the most tolerant of the minows tested against antimycin A. It required 100 p.p.b. for complete kills within 24 hours at 12°, but only 2 p.p.b. were needed for kills within 96 hours. Higher temperatures contributed to greater toxicities, and all goldfish perished within 96 hours when exposed to 1 p.p.b. at 17 and 0.6 p.p.b. at 22°.

Stonerollers were among the more sensitive minnows. Concentrations of toxicant as low as 1 **p.p.b.** killed all specimens within 24 hours at **12** , but variations in susceptibility were observed; a concentration which killed on one occasion failed on the next.

The golden shiner and fathead minnow were somewhat similar to the stoneroller in sensitivity, but all-or-none effects were delineated within a narrow range of concentrations. The golden shiners succumbed to 0.6 p.p.b. **within** 96 hours at 12°, and survival was noted at 0.05 p.p.b. Fathead minnows died at 0.4 p.p.b. and survived at 0.08 p.p.b.

Fresh-water catfishes

The catfishes were significantly less sensitive to antimycin A than other families (table 8). Channel catfish were more susceptible than bullheads. They survived 24-hour exposures at **12** to 2 p.p.b. but perished at 20 p.p.b. All specimens died at 6 p.p.b. in 96-hour tests at **22** .

The black bullhead was the more tolerant to the toxicant, and the yellow bullhead was only slightly less so. Concentrations of 120 and 100 p.p.b. respectively were required for complete kills in 24 hours at 12 . These concentrations are more than 100 times greater than those needed to kill fish of the most sensitive families.

The bullheads were affected by somewhat smaller quantities of chemical at 17 . Nevertheless, black bullheads tolerated 4 p.p.b. for 24 hours at 22°, and all died at 40 p.p.b.

TABLE 8.--Concentrations of antimycin A which caused all-or-none survival among black bullhead, yellow bullhead, and channel catfish at selected water temperatures in 24 hours and 96 hours

Species	Number of fish	Temperature (° C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Black bullhead.	848	12	10.0	120.0	10.0	80.0
Do........	120	17	6.0	60.0	4.0	40.0
Do........	120	22	4.0	40.0	2.0	40.0
Yellow bullhead.	192	12	20.0	80.0	20.0	80.0
Do........	84	17	<10.0	60.0	--	--
Channel catfish.	120	12	2.0	20.0	2.0	20.0
Do........	120	17	1.0	10.0	1.0	10.0
Do........	180	22	0.8	6.0	0.8	6.0

Other animals

Four hundred water fleas were used in trials with antimycin A. At 12 C., specimens survived 1 and 0.5 p.p.b., but died in 100 p.p.b. in 24 hours and in 10 p.p.b. in 48 hours. Their susceptibility increased with temperature. At 22°, they survived 0.1 p.p.b., but died in 10 p.p.b. in 24 hours and in 0.5 p.p.b. in 48 hours.

There were no mortalities among 20 crayfish exposed to 10 p.p.b. of toxicant at 12° for 96 hours.

Tests with 120 damselfly nymphs disclosed that the insects were relatively tolerant to antimycin A. At 12 , specimens survived 100 and 50 p.p.b. for 24 and 48 hours respectively, and 1,000 and 500 p.p.b. were required to kill them in the same time periods. At 22 , they survived 50 and 10 p.p.b., but died at 500 and 100 p.p.b. in 24 and 48 hours. The observations were not continued to 96 hours because high mortalities began to occur among controls.

Ninety-six tiger salamanders were exposed to antimycin A at 12 . Specimens survived 80 p.p.b. for 96 hours, but were killed by 600 p.p.b.

Among the 40 bullfrog tadpoles tested for 24 hours at 12 , the individuals exposed to 20 p.p.b. of toxicant survived whereas those subjected to 40 p.p.b. perished.

RESULTS OF FIELD STUDIES

Tests in wading pools

Results in 1962.--Some preliminary bioassays were conducted in 18 pools in July and October, to determine the utility of the pools as bioassay vessels and to yield information on the performance of antimycin A outdoors. A shortage of toxicant limited the scope of the trials, and a scarcity of fish of desirable species, sizes, and condition affected their validity. A number of the species were wild fish which later proved to be unsatisfactory test animals because of variable sizes, heavy parasitism, and poor condition.

The wading pools worked well as bioassay vessels. Fish, invertebrates, and plants did well in the test units and controls. There were some differences in the quantity of plankton and aquatic vegetation in the sand- and loam-bottom units because the latter were more fertile. The abundance of plants, we believe, contributed to increases in pH and alterations of alkalinity, and these in turn influenced the efficacy of antimycin A.

Goldfish, golden shiner, black bullhead, bluegill, largemouth bass, and yellow perch were exposed to 5 and 10 p.p.b. of toxicant in July. Most of them survived in the sand pools. The mortality was greater in the loam pools, especially at 10 p.p.b., but in no instance did it reach 100 percent. The black bullheads exhibited high tolerance to the toxicant in all pools.

Another series of tests was made in October with higher concentrations against rainbow trout, goldfish, golden shiner, bluntnose minnow, yellow bullhead, green sunfish, and yellow perch (table 9). The pH values in the pools at the time ranged from 7.5 to 9.9. Ninety to 100 percent of the trout, golden shiner, bluntnose minnow, green sunfish, and perch, and 60 percent of the goldfish were killed by 20 p.p.b. over sand and loam bottoms. At 40 p.p.b., there was very low survival among the trout, goldfish, and sunfish, but nearly complete survival of bullheads.

There appeared to be rapid degradation and detoxification of antimycin in the pools within 24 to 96 hours, depending on the initial concentration and the pH. Small numbers of goldfish, golden shiner, bluntnose minnow, bluegill, and largemouth bass were stocked later in pools in which antimycin A had been present for 24 to 72 hours. No more than half of the golden shiners and bluegills perished within the following 2 days.

Results in 1963.--The plants, plankton, and bottom fauna were permitted to develop in the pools for 2 months before toxicity trials. In July, acetone solutions of antimycin A were tested at 10, 20, 40, and 80 p.p.b. against eight species of fish of various sizes (tables 10, 11, 12, and 13). Golden shiners, bluegills, largemouth bass, and yellow perch were the more

TABLE 9.--Toxicity [illegible] antimycin A [illegible] 20 [illegible] 40 [illegible] sand- [illegible] loam-bottom [illegible]

[Mortalities [illegible]]

Species [illegible]	Type [illegible]	Antimycin A [illegible] 20 [illegible]					Antimycin A [illegible] 40 [illegible]				
		N[illegible]	N[illegible] (hours)--				N[illegible]	Number [illegible] in (hours)-			
			24	48	96	336		24	48	96	336
Adults:											
R[illegible]	[illegible]	20	16	16	17	19	20	16	17	18	20
Do............................	[illegible]	20	8	12	15	18	20	14	16	17	19
Y[illegible]	sand	40	0	0	0	1	40	0	0	0	1
Do............................	[illegible]	40	0	0	0	3	40	0	0	0	0
G[illegible]	[illegible]	40	0	0	7	37	40		1	12	40
Do............................	[illegible]	40	0	0	5	39	40	2	2	11	39
Fingerlings:											
G[illegible]	sand	40	1	11	17	25	40		21	37	37
Do............................	[illegible]	40	1	7	16	26	40	a	40	--	--
Golden [illegible]	sand	40	35	39	40	--	40	40	--	--	--
Do............................	[illegible]	40	37	40	--	--	40	40	--	--	--
Bluntnose [illegible]	[illegible]	40	33	39	40	-	40	40	--	--	--
Do............................	[illegible]	40	31	39	40	--	40	40	--	--	
Y[illegible]	[illegible]	40	40	--	--	--	40	40	--	--	--
Do............................	loam	40	40	--	--	--	40	40	--	--	--

TABLE 10.--Numbers [illegible] A [illegible] pools [illegible] July 1963

S[illegible]	T[illegible]	A[illegible] ([illegible])
G[illegible]	180	1.0
C[illegible]	180	2.2
G[illegible]	108	1.0
B[illegible] bullhead	180	2.0
D[illegible]	180	18.0
G[illegible]	144	2.7
B[illegible]	270	0.8
D[illegible]	180	22.0
Largemouth [illegible]	144	1.5
Y[illegible]	270	2.5

[illegible], [illegible] 10 [illegible]. within 48 [illegible]. C[illegible] 10 p.p.b. [illegible] 24 [illegible] 20 [illegible]. in [illegible]. S[illegible] 20 [illegible]., [illegible]- [illegible] 40 [illegible]. A[illegible] 80 [illegible]. [illegible] 48 [illegible]-[illegible].

T[illegible]- [illegible] 2 [illegible]

TABLE 11.--Toxicity [illegible] antimycin A at 10 and 20 [illegible]-[illegible] loam-bottom [illegible]

[Mortalities [illegible]]

S[illegible]	Type of [illegible]	Antimycin A [illegible] 10 [illegible]					A[illegible] A [illegible] 20 [illegible]				
		N[illegible]	N[illegible] ([illegible] --				Number [illegible] fish	N[illegible] ([illegible])--			
			24	48	96	480		24	48	96	480
A[illegible]:											
B[illegible]	[illegible]	20	0	0	0	2	20	0	0	0	0
D[illegible]	[illegible]	20	0	0	0	1	20	0	0	0	3
B[illegible]	[illegible]	20	17	20	--	--	20	20	--	--	--
Do...........................	[illegible]	20	20	--	--	--	20	20	--	--	--
F[illegible]:											
G[illegible]	[illegible]	20	6	6	6	6	20	20	--	--	--
D[illegible]	[illegible]	20	7	7	7	7	20	10	10	10	10
C[illegible]	[illegible]	20	14	14	14	14	20	20	-	--	--
D[illegible]	[illegible]	20	20	--	--	--	20	14	14	14	14
G[illegible]	[illegible]	14	14	--	--	--	14	14	--	--	--
D[illegible]	[illegible]	14	14	--	--	--	14	14	--	--	--
B[illegible]	[illegible]	20	0	0	0	0	20	0	0	0	0
D[illegible]	[illegible]	20	0	0	0	0	20	0	0	0	0
G[illegible]	[illegible]	16	15	15	15	15	16	16	--	--	--
Do...........................	[illegible]	16	16	--	--	--	16	16	--	--	--
B[illegible]	[illegible]	40	40	--	--	--	40	40	--	--	--
Do	[illegible]	40	40	--	--	--	40	40	--	--	--
Largemouth [illegible]	[illegible]	16	16	--	--	--	16	16	--	--	--
D[illegible]	[illegible]	16	16	--	--	--	16	16	--	--	--
Y[illegible]	[illegible]	20	20	--	--	--	20	20	--	--	--
D[illegible]	[illegible]	20	18	20	--	--	20	20	--	--	--

TABLE 12.--Toxicity of antimycin A at 40 and 80 p.p.b. on adult and fingerling fish in sand- and loam-bottom wading pools

[Mortalities are cumulative by observation period]

Species	Type of bottom	Antimycin A at 40 p.p.b.					Antimycin A at 80 p.p.b.				
		Number of fish	Number dead in (hours)--				Number of fish	Number dead in (hours)-			
			24	48	96	480		24	48	96	480
Adults:											
Black bullhead	sand	20	0	0	0	2	20	6	20	--	--
Do	loam	20	0	0	0	1	20	1	2	2	3
Bluegill	sand	20	20	--	--	--	20	20	--	--	--
Do	loam	20	20		-	--	20	20	--	--	--
Fingerlings											
Goldfish	sand	20	20		-		20	20	--	--	--
Do	loam	20	20	-	-	-	20	20	--	--	
Carp	sand	20	20	-	-		20	20	--	--	--
Do	loam	20	20				20	20	--		--
Golden shiner	sand	14	14	-		-	14	14	--	--	--
Do	loam	14	14	--	--	--	14	14	--	--	
Black bullhead	sand	20	0	0	0	0	20	20	--	--	--
Do	loam	20	0	0	0	0	20	11	11	11	11
Green sunfish	sand	16	16	--	--	--	16	16	--	--	--
Do	loam	16	16	-			16	16	--	-	
Bluegill	sand	40	40			-	40	40		--	--
Do	loam	40	40	-			40	40	-	--	
Largemouth bass	sand	16	16	-			16	16	--	--	--
Do	loam	16	16	--	-		16	16	--	--	
Yellow perch	sand	20	20	--			20	20	--	--	--
Do	loam	20	18	20			20	14	20		--

TABLE 13.--Average values of analyses made on water from sand- and loam-bottom wading pools before and after applications of antimycin A in July 1963

Item	Unit of measurement	Sand		Loam	
		Before	After	Before	After
Temperature	°C	23	25	23	27
Resistivity	at 20°C	2803	2864	3037	3052
Dissolved oxygen	p.p.m.O_2	8.7	9.1	9.7	9.7
Carbon dioxide	p.p.m.CO_2	0.0	0.0	0.0	0.0
Hydrogen ion	pH	8.8	9.1	8.8	9.2
Total alkalinity	p.p.m.$CaCO_3$	204.4	181.2	198.2	183.7
(as phenolphthalein)		(10.7)	(11.5	(14.6)	(14.3)
(as methyl orange)		(193.7)	(169.7)	(183.6)	(169.4)
Total hardness	p.p.m.$CaCO_3$	211.8	176.0	210.6	182.0
Calcium hardness	p.p.m.$CaCO_3$	53.6	47.9	60.0	53.6
Total iron	p.p.m.Fe	0.0	0.0	0.0	0.0
Sulfate ion	p.p.m.SO_4	25.8	13.4	18.3	11.1
Total phosphorus	p.p.m.PO_4	0.059	0.071	0.082	0.106
Ammonia nitrogen	p.p.m.NH_3	0.399	0.730	0.710	1.100
Nitrite nitrogen	p.p.m.NO_2	0.006	0.013	0.005	0.018
Nitrate nitrogen	p.p.m.NO_3	0.117	0.191	0.154	0.220
Chloride ion	p.p.m.CL	10.5	16.5	10.2	15.1

after exposure to the toxicant, and they exhibited a narcosislike condition. They showed little response to motion stimulus or handling with a dip net. Some of the larger bullheads behaved as if in distress and were subject to development of an unidentified funguslike condition on the body prior to death.

The trials in October included two formulations of antimycin. One was a solution in acetone, and the other an emulsifiable concentrate, applied to pools at 1, 5, 10, and 100 p.p.b. against 10 species of fish. The pH values at the time in all pools were about 10, and the antimycin A degraded so rapidly that most fish escaped toxic effects (table 14). The

TABLE 14.--Average values of analyses made on water from sand- and loam-bottom wading pools before and after applications of antimycin A in October 1963

Item	Unit of measurement	Sand		Loam	
		Before	After	Before	After
Temperature	°C	16	16	16	15
Resistivity	at 20°C	3561	3431	3439	3396
Dissolved oxygen	p.p.m.O_2	10.0	9.6	10.0	9.5
Carbon dioxide	p.p.m.CO_2	0.0	0.0	0.0	0.0
Hydrogen ion	pH	10.0	10.0	10.0	9.8
Total alkalinity	p.p.m.$CaCO_3$	114.0	107.0	127.0	121.0
(as phenolphthalein)		(29.0)	(27.5)	(36.0)	(34.0)
(as methyl orange)		(85.0)	(79.5)	(91.0)	(88.0)
Total hardness	p.p.m.$CaCO_3$	143.0	148.0	155.0	154.0
Calcium hardness	p.p.m.$CaCO_3$	27.4	33.0	38.0	35.0
Total iron	p.p.m.Fe	0.025	0.026	0.036	0.028
Sulfate ion	p.p.m.SO_4	17.8	15.3	18.0	14.0
Total phosphorus	p.p.m.PO_4	0.090	0.084	0.043	0.035
Ammonia nitrogen	p.p.m.NH_3	0.25	0.270	0.000	0.550
Nitrite nitrogen	p.p.m.NO_2	0.0	0.0	0.0	0.0
Nitrate nitrogen	p.p.m.NO_3	0.0	0.0	0.0	0.0
Chloride ion	p.p.m.Cl	12.6	15.25	11.0	13.6

exceptions were those individuals exposed to 100 p.p.b. It appeared that the acetone solution of toxicant deteriorated sooner than the other preparation.

Of the 10 species of fish, 7 species succumbed totally to 100 p.p.b. of acetone-antimycin A, and 9 species to the emulsifiable formulation, within 24 hours over sand bottoms; only carp, fathead minnow, bluegill, longear sunfish, and yellow perch died over loam bottoms. The black bullhead was the sole survivor of 100 p.p.b. over both bottom types. Neither preparation of toxicant caused 100-percent kills of any species within 96 hours at 5 or 10 p.p.b.

In general, most of the susceptible fish showed signs of distress within a short time after exposure, and many came to the surface of the pools. The length of time which elapsed before death varied with the species and water temperature, and ranged from a few hours to several days. It is significant that all specimens which displayed symptoms of distress eventually died. This suggests that the action of the toxicant on fish is irreversible.

There were no grossly toxic effects by antimycin A on the plankton, bottom fauna, or aquatic plants during the course of the July and October trials. For example, in the four pools which received 20 p.p.b. of antimycin A in July, the average quantity of plankton was 0.0036 cc./l. (range: 0.0020 to 0.0044) before treatment and 0.0040 cc./l. (range: 0.0033 to 0.0061) at 20 days after treatment. The quantities in two control pools were 0.0047 and 0.0089 cc./l. during pretreatment sampling and 0.0022 and 0.0044 cc./l. during post-treatment sampling.

Tests in hatchery ponds

There appeared to be a more rapid response of fish to the antimycin A which was formulated with an emulsifiable concentrate than with acetone. With the former preparation in pond No. 2, fish surfaced within 4 to 6 hours after application, whereas in pond No. 5 there were no comparable effects for another 10 hours. By the end of the first full day, we saw no significant differences in the effects produced by the two formulations. Table 15 gives before and after water analyses for the two ponds.

Northern pike were the first fish to exhibit distress. They surfaced and appeared to be in a state of narcosis which was followed by complete locomotor ataxia. The rainbow trout, white suckers, carp, walleye, and sunfishes followed in order with similar symptoms. The great majority of specimens were dead within 48 hours (tables 16 and 17). It is noteworthy that goldfish--a species which was relatively

TABLE 15.--Analyses of water from ponds No. 2 and No. 5 at Delafield Warmwater Fisheries Research Station before and after applications of antimycin A in September 1963

Item	Unit of measurement	Pond No. 2		Pond No. 5	
		Before	After	Before	After
Temperature..........	°C	21	15	21	17
Resistivity..........	at 20°C	2550	2600	2525	2600
Dissolved oxygen.....	p.p.m.O_2	6.7	6.9	7.5	8.2
Carbon dioxide.......	p.p.m.CO_2	3.4	0.0	2.0	0.0
Hydrogen ion.........	pH	8.0	8.4	8.9	8.5
Total alkalinity.....	p.p.m.$CaCO_3$	210.0	202.0	201.1	189.5
(as phenolphthalein)	....	(0.0)	(0.0)	(8.8)	(0.0)
(as methyl orange)..	....	(210.0)	(202.0)	(192.3)	(189.5)
Total hardness.......	p.p.m.$CaCO_3$	213.0	220.0	202.0	208.0
Calcium hardness.....	p.p.m.$CaCO_3$	77.0	82.0	80.0	75.0
Manganese............	p.p.m.Mn°	0.0	0.0	0.0	0.0
Total iron...........	p.p.m.Fe°	0.00	0.05	0.00	0.13
Sulfate ion..........	p.p.m.SO_4	44.3	38.0	39.0	35.0
Total phosphorus.....	p.p.m.PO_4	1.40	0.10	0.50	0.15
Ammonia nitrogen.....	p.p.m.NH_3	0.20	0.19	0.18	0.38
Nitrite nitrogen.....	p.p.m.NO_2	0.0	0.0	0.0	0.0
Nitrate nitrogen.....	p.p.m.NO_3	0.07	0.50	0.07	0.43
Chloride ion.........	p.p.m.CL				

TABLE 16.--Effects of 10 p.p.b. of antimycin A in emulsifiable concentrate on 18 species of fish in pond No. 2

Species	Total fish stocked	Average length (inches)	Average weight (grams)	Number of fish dead at (hours)--			
				24	48	96	480
Longnose gar.....	3	25.6	658	0	0	0	0
Bowfin...........	1	16.8	545	0	0	0	0
Rainbow trout....	312	4.0	82				-
Northern pike....	7	17.8	713	5	5	5	7
Goldfish.........	740	2.4	9	740	--	--	--
Carp.............	18	15.3	1,126	17	18	--	--
White sucker.....	4	15.1	554	3	3	3	[illegible]
Black bullhead...	600	3.8	18	0	0	0	[illegible]
Yellow bullhead..	4	8.3	168	0	0	0	0
Brown bullhead...	1	4.2	50	0	0	0	0
Rock bass........	1	8.0	136				-
Green sunfish...	3	3.8	14	0	0	1	3
Pumpkinseed......	13	4.6	41	11	12	13	
Bluegill.......	27	6.1	68	21	21	22	
Black crappie....	7	8.3	95	5	5	5	
Largemouth bass..	4	15.4	795	1			
Hybrid sunfish...	1,400	1.7	9	1,400	--	--	
Walleye..........	1	13.5	318				

TABLE 17.--Effects of 10 p.p.b. of antimycin A in acetone solution on 19 species of fish in pond No 5

Species	Total fish stocked	Average length (inches)	Average weight (grams)	Number of fish dead at (hours) --			
				24	48	96	480
Longnose gar....	3	24.6	395	0	0	2	2
Bowfin.........	1	21.8	1,771	0	0	0	0
Rainbow trout...	470	4.1	86	470	--	-	--
Northern pike...	8	19.1	976	6	6	8	--
Goldfish.......	1,400	2.7	9	1,400	--	--	--
Carp...........	27	15.3	1,112	26	27	--	--
White sucker....	6	15.7	636	5	5	5	6
Black bullhead..	875	3.7	14	0	2	3	157
Yellow bullhead..	1	5.7	59	0	0	0	0
Brown bullhead..	6	11.4	377	0	0	0	1
Rock bass.......	1	8.2	136	0	0	0	1
Green sunfish...	4	4.2	23	0	1	2	4
Pumpkinseed.....	24	4.6	32	2	12		24
Bluegill.......	43	6.4	86	19	28		43
Black crappie...	9	8.8	136	1	4		9
Largemouth bass..	5	12.8	477	0	2		5
Hybrid sunfish..	2,055	1.7	9	2,055	--		--
Walleye........	2	13.0	386				--
Drum...........	1	11.6	272	0	0		--

TABLE 18.--Abundance of bottom fauna in ponds No. 2 and No. 5 before and after treatment with 10 p.p.b. of antimycin A

[Each collection consisted of 16 one-square foot samples

Organism		Average number per square oot			
		Pond No. 2		Pond o. 5	
		Sept. 23	Oct. 15	Sept. 17	Oct. 14
Horsehair worm	(Nematomorpha)..	10.7	0.7	1.0	1.0
Aquatic earth-worm	(Oligocheata)...	0.0	0.0	34.5	3.0
Leech	(Hirundinea)....	0.0	1.3	5.2	1.0
Scuds	(Amphipoda).....	4.7	2.0	17.5	39.0
Mayflies	(Ephemeroptera..	9.0	145.3	6.8	6.5
Damselflies	(Zygoptera).....	1.7	2.0	6.8	7.8
Dragonflies	(Anisoptera)....	0.0	0.7	0.5	0.5
Waterbugs	(Hemiptera).....	0.0	2.7	1.0	1.5
Caddisflies	(Trichoptera)...	0.7	2.0	0.2	0.0
Water beetles	(Coleoptera)....	2.7	18.0	5.0	3.5
Mosquitoes	(Culicidae).....	0.0	0.0	0.8	0.0
Midges	(Tendipedidae)..	209.7	388.0	269.5	422.0
Biting midges	(Ceratopogonidae)..	1.3	0.0	2.8	1.0
Soldierflies	(Stratiomyiidae)...	0.3	0.0	0.0	0.0
Snails	(Gastropoda).....	4.0	30.7	72.8	30.5
	Total..........	244.8	593.4	424.4	517.3

tolerant to antimycin A in the laboratory--died in both ponds within 24 hours.

The longnose gar, bowfin, black bullhead, yellow bullhead, and brown bullhead were the only species which were not affected greatly by the toxicant at 10 p.p.b. Seventy percent of them were recaptured alive when the ponds were drained after 20 days.

The detoxification of antimycin A was monitored throughout the first 96 hours. It occurred within 72 hours after application, and fish placed in live cages after this time survived until the ponds were drained.

Plankton was sampled in both ponds during the experimental period. In pond No. 2, the pretreatment quantity was 0.018 cc./1 and the posttreatment quantity was 0.047 cc./1. Pond No. 5 had pretreatment and posttreatment quantities of 0.0035 and 0.039 cc./1. None of the relatively minor changes was attributed to the toxicant. Also, there were no observable changes in the aquatic plants in the ponds.

Pretreatment and posttreatment samples of bottom fauna were taken. We concluded that antimycin A was nontoxic to the 15 taxonomic groups which were represented in both ponds because there were no significant changes in species composition or numerical abundance (table 18). The midges were the more numerous in all samples, and they increased by 55 to 65 percent during the experimental period. The nymphs of mayflies, dragonflies, and damselflies were also more abundant in the posttreatment samples.

Care was taken to note any gross effects of the toxicant on frogs, salamanders, and turtles, but there were none.

Discussion of field studies

There was a lack of consistency in the performance of antimycin A in sand- and loam-bottom pools in July and October, 1962 and 1963, and in the hatchery ponds. The cause, we believe, was the chemistry of the waters and particularly the presence of the hydroxyl ion.

An alkaline shift occurred in the wading pools as the plant biomass increased. The relatively hard, well water which was used to fill the pools was gradually softened because of the decrease in calcium. There was a shift from bicarbonate (methyl orange alkalinity) to free hydroxide (phenolphthalein alkalinity). The measure of the acid-base shift was pH which rose from 7.5 upward to 10 or more. Diurnal fluctuations of several pH units are not uncommon in ponds, and the pH in wading pools ranged accordingly between morning and afternoon.

The highest pH values were observed in late afternoon in the presence of abundant plants and sunshine. In this situation, the hydroxyl ions appear, and often they are not checked by buffering salts. Magnesium prevails as calcium ions are removed from solution, and the result is the sort of alkaline shift observed in softer waters.

We assume that the relative success of the toxicity trials in hatchery ponds was due in large part to the fact that the water had high buffer capacity and little reserve alkalinity in the form of hydroxide. Thus, the antimycin A was not subject to immediate detoxification by action of free hydroxide, and the 10 p.p.b. were effective in killing fish.

In contrast, the poorer results obtained in the wading pools reflected the greater concentrations of free hydroxide present. In July 1963, the pools had approximately the same pH and total alkalinity as the hatchery ponds, but there was more free hydroxide present. Therefore, the degradation of the toxicant was more rapid, and 20 to 40 p.p.b. were needed to kill fish.

The contrast was heightened by results in October 1963. The water was much softer and lower in buffer capacity, and there was even more free hydroxide present. The pH ranged up to 10. Under these conditions, there was almost immediate detoxification of the antimycin, and only partial fish kills were obtained at 100 p.p.b.

CONCLUSIONS

Antimycin A is a powerful toxicant to fresh-water fish. We observed the responses of many specimens to concentrations which ranged from 0.01 to 120 p.p.b. Among them, the carp--a most undesirable fish in many waters--proved vulnerable to small concentrations and short exposures at cool and warm temperatures. Other fishes which at times may be undesirable, such as goldfish, white suckers, green sunfish, and pumpkinseeds, were also killed.

The sensitivities to antimycin A varied among species, and they were correlated with temperature and duration of exposure. The tests in the laboratory at 12°, 17°, and 22° C. indicated that smaller quantities of toxicant or shorter exposures produced kills of fish in warmer waters, but the results at 12° were nonetheless satisfactory.

There were three general degrees of sensitivity detected among the 24 species of fish in the laboratory and a similar order among the 25 species used in outdoor trials. Indicative of the extremes in response, gizzard shad perished at 0.04 p.p.b. of toxicant whereas black bullheads survived 100 p.p.b. There also appeared to be a tendency for sensitivities to follow family lines, but species in the nine families tested exhibited great variations in susceptibility. For example, fingerling carp in the laboratory died within 24 hours upon exposure to 0.6 p.p.b. at 12°, but up to 100 p.p.b. were required for complete kills of goldfish.

Observations in the laboratory and field demonstrated that antimycin A was less toxic to other animals. Water fleas were killed by 100 p.p.b. in 24 hours at 12°, but their susceptibility increased at warmer temperatures or longer exposures. Crayfish were not harmed by 10 p.p.b. over 96 hours, and damselfly nymphs survived 50 p.p.b. for 48 hours. Tiger salamanders survived 80 p.p.b. for 96 hours at 12°, and bullfrog tadpoles were unharmed by 20 p.p.b. for 24 hours.

The plankton in wading pools and hatchery ponds was not significantly affected during experiments, and there was no gross evidence of toxicity to filamentous algae, and submersed and emergent plants. No deleterious effects were detected on the composition, numbers, and growth of bottom fauna in hatchery ponds.

Antimycin A degraded rapidly in water, and detoxification was complete within 24 to 96 hours under field conditions. The rate of molecular breakdown was accelerated sharply in the presence of free hydroxide, and this suggests a possibility for artificial detoxification. Bioassays with fish following the degradation of the toxicant revealed an absence of harmful residues in water.

Further investigation on antimycin A as a fish toxicant is warranted in the laboratory and field. Studies are contemplated or in progress at the Fish Control Laboratories on its performance against various life stages of fish from egg to adult; against additional species; on minimum killing concentrations and exposures; in waters of various chemistries; and at cold and warm temperatures. Appropriate formulations for standing and flowing waters are desirable. Further attention must also be given to the effects of the toxicant on other aquatic organisms. The factors in water which contribute to degradation of the toxicant deserve study, and the nature and fate of residues require definition. Also--and depending on adequate supplies of toxicant--m any, and more comprehensive, trials in the field are needed for full and fair evaluation of this material which has potential value in fishery management and research.

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