

Survival of Two Species of Freshwater Clams,
Corbicula leana and *Magnonaias boykiniana*,
After Exposure to Antimycin

by

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Abstract

The Asiatic clam, *Corbicula leana* Prime, and a clam native to the southern United States, *Magnonaias boykiniana*, were exposed to the fish toxicant antimycin at several concentrations for various periods and then placed in an untreated earthen pond for posttreatment observation. Both species survived the concentrations and exposure periods usually used in field application. However, latent mortalities were observed in the pond 3 months after a 30-day flow-through exposure of *Corbicula* to 3.6 to 30 $\mu\text{g/l}$ of antimycin. A single treatment (2 $\mu\text{g/l}$) in an earthen pond did not result in significant mortalities of *Corbicula* during 22 weeks. *Magnonaias* was more sensitive than *Corbicula* to antimycin, but both survived the maximum permissible use-pattern concentrations in flow-through tests.

Asiatic clams (several species of the genus *Corbicula*) introduced into U.S. waters have spread to many important river systems (Sinclair and Isom 1963; Keup et al. 1963; Diaz 1974). Asiatic clams are becoming so numerous that they cause problems in water intakes to industrial plants, in the pipes and plumbing of municipal water supplies, in sand and gravel operations, and as competitors with native species of clams for habitat and food.

The widespread distribution of Asiatic clams suggests hardiness and an ability to tolerate adverse environmental conditions. Sinclair and Isom (1963) indicated that these clams can survive fluctuating environmental conditions, and Chandler and Marking (1975) reported that they are more resistant than native clams to the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM). Burress et al. (1976) found Asiatic clams useful in laboratory toxicity tests because they are hardy (*Corbicula leana* survived for 18 months in

outdoor plastic pools with no water exchange) and can be easily collected, transported, handled, and exposed to chemicals.

Magnonaias boykiniana, the other clam used in the present studies, is native to many American river systems, including those in Georgia.

Pond studies with the fish toxicant, antimycin, showed that applications of 5 $\mu\text{g/l}$ severely reduced plankton populations, whereas benthic invertebrates were not severely reduced (Callahan 1968; Callahan and Huish 1968). Field applications of antimycin in Wisconsin were reported to have resulted in delayed mortality of several species of mollusks in the East Branch, Rock River (Bratley and Mathiak 1972) and in the Ashippum River (Flowers et al. 1975). A comprehensive summary of data on the effects of antimycin on nontarget organisms prepared by Schnick (1974) indicated that the 96-h LC_{50} for antimycin against Asiatic clams was 50 $\mu\text{g/l}$.

Antimycin is generally applied at concentrations ranging from 1 to 10 $\mu\text{g/l}$ in single applications for the control of undesired fish populations in ponds and lakes, or for up to 12 h in streams. The present study was designed to determine the acute toxicity and latent mortality resulting from short- and long-term exposures of the Asiatic clam, *Corbicula leana*, and a native clam, *Magnonaias boykiniana*, to antimycin. Concentrations and exposures investigated equaled or exceeded those currently used for fish control.

Materials and Methods

The clams used in this study were collected from shoals of the Flint River east of Woodbury (Upson County), Georgia. They were transported to the laboratory and kept in limed flowing water until exposed to the toxicant. Deformed organisms or any that appeared weakened by handling were discarded. Mature specimens of uniform size were selected for the tests.

Clams were exposed to antimycin (Fintrol Concentrate formulation) in limed spring water (total hardness 18–22 mg/l as CaCO_3 , and pH 6.8) in four types of tests.

(1) Standardized static laboratory tests were conducted in three series of 19-liter glass jars containing 15 liters of water. The substrate was mud in one series and sand in a second; one series of jars contained no substrate. Ten Asiatic clams were exposed to 5, 10, or 20 $\mu\text{g/l}$ of antimycin for 1 or 30 days. Antimycin was prepared in concentrated stock solutions and portions were added to the test water to obtain desired concentrations. Tests were conducted according to recommendations of the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Surviving clams were placed in cages in untreated, previously filled earthen ponds for observation over a 112-day period.

(2) An earthen 0.04-ha pond was treated with 2 $\mu\text{g/l}$ of antimycin by dispensing a solution of the toxicant into the propeller wash of an outboard motor. Mortality was recorded among the 300 stocked Asiatic clams and 85 native clams (weight range, 200–500 g) over a 22-week posttreatment observation period. The pond water had the following characteristics: total hardness as CaCO_3 , 2.0–14.0 mg/l; pH 5.6–7.4; temperature 6.0–19.0 C; and dissolved oxygen 8.7–15.0 mg/l.

(3) Standardized flow-through toxicity tests were conducted in an apparatus similar to that described by Mount and Brungs (1967). Asiatic clams were exposed to 3.6 to 30 $\mu\text{g/l}$ of antimycin in 50-liter glass aquaria for 30 days. Thirty clams were exposed to each concentration. The stock solution of antimycin was metered into a mixing box, and dilutions of the mixture yielded

selected concentrations. Mortalities were recorded daily during exposure.

(4) Flow-through tests were conducted in fiberglass tanks in which 300 Asiatic and 75 or 85 native clams were exposed to 0.2 and 5 $\mu\text{g/l}$ of antimycin for 12 h or to 50 $\mu\text{g/l}$ for 24 h. After exposure, the clams were observed in untreated pond water for 22 weeks. Antimycin solutions were mixed in epoxy-coated steel tanks containing 3,623 liters of limed spring water and pumped through the tanks at a rate of 4.4 liters/min.

Small amounts of boiled trout chow and cereal leaves (*Daphnia* food) were offered weekly to the Asiatic clams in long-term static and flow-through exposures, and excess food was routinely siphoned off.

Survivors of each test were acclimated to holding pond temperatures and placed in cages submersed in the ponds. Each cage contained a 2-cm layer of soil substrate obtained from the holding pond. Cages were placed so that water continuously covered the substrate in the cage to a depth of 41 to 46 cm.

The clams were moved to the holding ponds and examined semimonthly after exposure. Clams that were unable to retract the foot or adduct the valves were considered dead. The period between mortality determinations was usually long enough so that only a casual observation was required because the soft tissues of dead specimens protruded from the valves. All mortality tabulations are cumulative.

Results

Corbicula survived 1-day exposures to 5, 10, and 20 $\mu\text{g/l}$ of antimycin in 19-liter glass jars containing no substrate or substrates of sand or mud (Table 1). During the 29-day posttreatment holding period in jars containing untreated water, no clams died that had been exposed with no substrate, or with mud substrate; however, 70% of the clams died that had been exposed to 20 $\mu\text{g/l}$ of antimycin in jars with sand substrate. During the later 112-day period in the holding pond, a few more died that had been exposed in jars with a sand substrate.

Clams exposed for 30 days to 5, 10, or 20 $\mu\text{g/l}$ of antimycin were affected especially during the posttreatment observation period. By the end of the 112-day holding period in earthen ponds, all clams had died, except for 4 of 10 exposed to 5 $\mu\text{g/l}$ with a sand substrate; thus exposure time was a critical factor.

Antimycin was generally not toxic in 1-day exposures, but latent mortality developed in the 30-day exposures. The 1-day exposures typify field use patterns, and 30-day exposures greatly exceed those used in fishery management.

Table 1. Cumulative percentage mortality of *Corbicula leana* after static exposures of 1 day or 30 days to selected concentrations of antimycin. Each jar contained 10 clams.

Substrate and concentration of antimycin ($\mu\text{g/l}$)	Exposure period (days)	Days in jars ^a	Days in untreated holding pond after exposure			
			28	70	112	
No substrate						
0	1	0	0	0	0	
0	30	0	0	10	10	
5	1	0	0	0	0	
5	30	10	30	80	100	
10	1	0	0	0	0	
10	30	50	80	100	100	
20	1	0	0	0	0	
20	30	50	60	100	100	
Sand						
0	1	0	0	0	10	
0	30	0	0	0	0	
5	1	0	0	10	10	
5	30	0	20	50	60	
10	1	10	10	20	20	
10	30	10	20	90	100	
20	1	70	70	70	70	
20	30	20	20	90	100	
Mud						
0	1	0	0	0	0	
0	30	10	10	10	10	
5	1	0	0	0	0	
5	30	20	20	100	100	
10	1	0	0	0	0	
10	30	50	50	70	100	
20	1	0	0	0	0	
20	30	40	40	100	100	

^aClams exposed for 1 day were placed in 15 liters of untreated water in 19-liter jars for 29 days after exposure, and then placed in a holding pond with those exposed in jars for 30 days.

In the static pond application, in which *Corbicula* and *Magnonaias* were exposed to 2 $\mu\text{g/l}$ of antimycin and the toxicant was allowed to dissipate and detoxify with time, 98% of the *Corbicula* and nearly 65% of the *Magnonaias* survived for 22 weeks after the toxicant application (Table 2).

Table 2. Cumulative percentage mortality after 2 to 22 weeks among 300 *Corbicula leana* and 85 *Magnonaias boykiniana*, in a pond treated with 2 $\mu\text{g/l}$ of antimycin.

Species	Weeks			
	0	8	16	22
<i>Corbicula</i>	0	1.7	1.7	2.0
<i>Magnonaias</i>	0	22.4	32.9	35.3

Corbicula survived 30 days of exposure to antimycin concentrations of 3.6 to 30 $\mu\text{g/l}$ in a standardized flow-through system (Table 3). Exceptions were a single mortality at 3.6 $\mu\text{g/l}$ and two at 15.1 $\mu\text{g/l}$. These mortalities probably resulted from stresses other than the antimycin, since all clams survived at higher concentrations. Few clams died during the first 86 days after they were transferred to the holding pond, but latent mortality became significant thereafter. After 156 days, mortality ranged from 27 to 77% (Table 3). The die-off seemed to stabilize toward the end of the observation period; the increase in mortality from 128 to 156 days was minor. Again, the 30 days of continuous exposure that led to latent mortality far exceeded exposure time in field applications.

Survival of *Corbicula* exposed to 2 and 5 $\mu\text{g/l}$ of antimycin for 12 h was high during exposure and during

Table 3. Mortality among 30 *Corbicula leana* during exposure for 30 days to various concentrations of antimycin in a flow-through system, and cumulative percentage mortality in a holding pond 44 to 156 days posttreatment.

Concentration ($\mu\text{g/l}$)	Percentage mortality during exposure	Days after end of exposure period			
		44	86	128	156
0.0	0.0	0.0	0.0	0	0
3.6	3.3	3.3	10.0	47	53
4.4	0.0	0.0	3.3	27	27
5.9	0.0	0.0	0.0	23	30
8.3	0.0	0.0	3.3	50	50
12.4	0.0	0.0	3.3	50	57
15.1	6.6	6.6	16.0	63	67
20.7	0.0	0.0	3.3	63	73
24.1	0.0	0.0	3.3	67	70
30.0	0.0	3.3	6.6	77	77

the later 22-week observation period in an earthen pond; mortality ranged from only 0.7 to 2.7% for exposed clams and was 2.3% for unexposed clams (Table 4). The mortality of *Corbicula* exposed to 50 $\mu\text{g/l}$ of antimycin for 24 h was slightly higher — 4% at 2 weeks and 8.7% after 22 weeks.

Magnonaias was more sensitive than *Corbicula* to antimycin in the 12-h exposures to 2 and 5 $\mu\text{g/l}$ and the 24-h exposure to 50 $\mu\text{g/l}$ in the flow-through tank system. Mortality was low among *Magnonaias* exposed for 12 h during the first 2 weeks of observation, but increased to 20 to 53% after 22 weeks (Table 4). During the 22-week period, however, 20% of the unexposed clams also died. The concentrations of 5 $\mu\text{g/l}$ of antimycin for 12 h and 50 $\mu\text{g/l}$ for 24 h killed more than half of the clams during the 22-week observation period. Despite these losses it seems clear that the population would not have been eliminated, even by 24-h exposures to 50 $\mu\text{g/l}$.

Discussion

Exposure times longer than 12 h and concentrations of antimycin higher than 10 $\mu\text{g/l}$ are rare in stream treatments with antimycin. The registered label prescribes concentrations of 1 to 10 $\mu\text{g/l}$, depending on physical and chemical conditions of the water. Our exposures of 30 days were excessive. In streams the toxicant is added to flowing water, where concentrations build up to a peak of short duration and then decrease as a result of dilution and decomposition. Stream organisms are thus exposed to a slug of the toxicant passing downstream for an exposure time that varies with the species of target fish, and with environmental conditions (Gilderhus 1972).

Table 4. Cumulative percentage mortality among 300 *Corbicula leana* and 75 or 85 *Magnonaias boykiniana* exposed to antimycin for 12 or 24 h in flow-through tanks and moved to an earthen pond for observation for 22 weeks.

Species and concentration ($\mu\text{g/l}$)	Exposure (h)	Weeks			
		2	8	16	22
<i>Corbicula</i>					
0	12	0.3	0.3	1.7	2.3
2	12	0.0	0.7	0.7	0.7
2	12	—	2.7	2.7	2.7
5	12	0.6	1.7	1.7	2.0
50	24	4.0	6.0	8.7	8.7
<i>Magnonaias</i>					
0	12	1.3	13.3	18.7	20.0
2	12	0.0	9.3	17.3	20.0
2	12	—	12.9	23.5	34.1
5	12	3.5	35.3	50.6	52.9
50	24	2.4	30.6	48.2	50.6

References

- Bratley, D. A., and H. A. Mathiak. 1972. Mussels. Pages 94-97 in U.S. Department of the Interior Draft Environmental Statements: Fishery rehabilitation of the Rock River, Dane, Dodge, Columbia, Fond du Lac, Green Lake, Jefferson, Rock, Washington, Walworth, and Waukesha Counties, Wisconsin. U.S. Fish Wildl. Serv., Washington, D.C. DES [Draft Environ. Statement] 72-77.
- Burress, R. M., J. H. Chandler, and L. L. Marking. 1976. Use of the Asiatic clam, *Corbicula leana* Prime, in toxicity tests. Prog. Fish-Cult. 38(1):10.

- Callaham, M. A. 1968. Antimycin as a fisheries tool in the Southeast. Ph.D. Thesis. University of Georgia, Athens. 76 pp.
- Callaham, M. A., and M. T. Huish. 1968. An evaluation of antimycin as a selective bluegill toxicant under varying conditions of pH. Proc. Annu. Conf. Southeast. Assoc. Game Fish Comm. 21(1967):476-481.
- Chandler, J. H., and L. L. Marking. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to selected aquatic invertebrates and frog larvae. U.S. Fish Wildl. Serv., Invest. Fish Control 62. 7 pp.
- Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Series. EPA [Environ. Prot. Agency]-660/3-75-009. 61 pp.
- Diaz, R. J. 1974. Asiatic clam, *Corbicula manilensis* (Philippi), in the tidal James River, Virginia. Chesapeake Sci. 15(2):118-120.
- Flowers, R. W., R. M. Johnson, and W. L. Hilsenhoff. 1975. Effect of antimycin on instream mussel populations. Wis. Dep. Nat. Resour. Res. Rep. 84. 11 pp.
- Gilderhus, P. A. 1972. Exposure times necessary for antimycin and rotenone to eliminate certain freshwater fish. J. Fish Res. Board Can. 29(2):119-202.
- Keup, L., W. B. Horning, and W. M. Ingram. 1963. Extension of range of Asiatic clam to Cincinnati reach of the Ohio River. Nautilus 77(1):18-21.
- Mount, D. L., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1:21-29.
- Schnick, R. A. 1974. A review of the literature on the use of antimycin in fisheries. FWS [Fish Wildl. Serv.] LR [Lit. Rev.] 74-01. NTIS [Natl. Tech. Info. Serv.] No. PB-235, 440/AS. 85 pp.
- Sinclair, R. M., and B. G. Isom. 1963. Further studies on the introduced Asiatic clam (*Corbicula*) in Tennessee. Tennessee Stream Pollution Control Board, Nashville. 78 pp.

