TOXICITY OF ANTIMYCIN A TO ASELLUS INTERMEDIUS, DUGESIA DOROTOCEPHALA, GAMMARUS PSEUDOLIMNAEUS, AND HYALELLA AZTECA

Paul C. Baumann
James W. A. Jaeger
Mary E. Antonioni
University Wisconsin — Madison

ABSTRACT

Antimycin A, a registered fish toxicant, was tested in the laboratory on Asellus intermedius, Dugesia dorotocephala, Gammarus pseudolimnaeus, and Hyalella azteca. H. azteca and G. pseudolimnaeus were very sensitive with 96 hr LC 50s < 10 μg/1. A. intermedius also showed mortality at this level in one series of experiments. D. dorotocephala showed no mortality at 15 μg/1 of Antimycin A for eight days. The 96 hr ECT 50 values at 10 μg/1 of Antimycin A were determined for G. pseudolimnaeus (1.4 hr) and H. azteca (5.3 hr). Based on these results, the 10 μg/1 level of Antimycin A normally used in fish control would probably eliminate G. pseudolimnaeus and H. azteca, two important fish food organisms.

INTRODUCTION

Antimycin A, a respiratory inhibitor registered as a fish toxicant in 1966, has received increasing use for rough fish control in lake and stream management. Much laboratory and field experimentation has been done with Antimycin A, as a piscicide (Berger et al., 1969; Gilderhus et al., 1969; Lennon and Berger, 1970; Marking and Dawson, 1972), but there is a lack of information on the toxicity of the compound to common invertebrates. In view of the use of the chemical for a large-scale fish removal project in the Rock River, Wisconsin, we were encouraged to conduct toxicity experiments with the amphipods Gammarus pseudolimnaeus Bousfield and Hyalella azteca (Saussure), the isopod Asellus intermedius Forbes, and the planarian Dugesia dorotocephala (Woodworth).

These species are abundant in portions of the Rock River system and are important fish foods. Gammarus spp. are important in the diet of a wide variety of gamefish species including brown trout (Reimers et al., 1955; and Maitland, 1965), brook trout (Rawson and
Elsey, 1950), and walleye (Kelso, 1973). *Asellus spp.* are abundant in the diets of warmouth and largemouth bass (Larimore, 1957) and brown trout (Ellis and Gowing, 1957). *H. azteca* occurs in the diets of black crappie and bluegill (Seaburg and Moyle, 1964) and largemouth bass (McCammon et al., 1964).

Another consideration in the choice of these test organisms is the fact of their totally aquatic life cycles. Repopulation would be difficult in waters where they were completely eliminated. Insect species with a winged adult stage for dispersal could more easily reinvade such streams and long-term eradication would be avoided.

Lennon and Berger (1970) reported invertebrate mortalities observed in field trials, i.e. nearly total kills (99%) of rotifers, cladocerans and copepods, with partial kills of fresh-water shrimp and *Gammarus spp.* In their discussion they indicate that fall frosts may have been responsible for the zooplankton decline and postulate that the partial kill of freshwater shrimp was due to locally high toxicant concentrations. They suggest that dosage levels of Antimycin A used for fish control (10-15 μg/l) do not ordinarily adversely affect aquatic invertebrates.

Recent studies on clams (Antonioni, 1974), ostracods (Kawatski, 1973) and caddis flies and *Gammarus* (Lesser, 1972) indicate that these animals suffer mortality at low dosage levels of Antimycin A (10-15 μg/l).

**EXPERIMENTAL PROCEDURE**

The formulation of Antimycin A used was Liquid Fintrol Concentrate (Ayerst Laboratories, Inc.). An initial stock solution was prepared in acetone to insure uniformity and provide a stable solution. The final mixing with water for the desired treatment dosages was done just prior to the start of each experimental run. The Antimycin A stock solution was mixed according to directions of the manufacturer to arrive at the desired dosage.

Individual experiments were conducted with two-liter glass vessels containing twelve organisms of a single species. These containers were placed in a water bath maintained at 15°C under constant light.

*A. intermedius* and *G. pseudolimnaeus* collected from the Bark River, Waukesha Co., Wis. were tested with two different types of water, Biotron tap water (Antonioni, 1974) and Bark River water collected with the organisms. In these tests, the water was aerated and the containers were covered with a plastic sheet to prevent
evaporation. Three Antimycin A concentrations between 5 and 15 μg/1 were employed to determine the concentration causing a 50% mortality (LC 50).

H. azteca from Lake Mendota, Dane Co., Wis., G. pseudolimnaeus from Parfrey's Glen Creek, Sauk Co., Wis.; and D. dorotocephala from Turtox-Cambosco Biological Supply Company were tested in Biotron tap water without aeration.

In addition, time series experiments were run with H. azteca and G. pseudolimnaeus. These animals were added to the toxicant at 10 μg/1 and were then removed, rinsed, and transferred to untreated water at various time intervals. This allowed determination of the time of exposure to cause a 50% mortality (ECT 50).

All experimental animals were acclimated for 24 hr in the laboratory prior to the experiment. Observations for death were made at 24 hr intervals, and dead organisms (those failing to respond to mechanical stimulation) were removed and held in fresh water for further observation to confirm death. Sick or weak individuals were not removed.

Mortalities at 96 hr were analysed by the graphical method of Litchfield and Wilcoxon (1949) to obtain LC 50 and ECT 50 values.

RESULTS

H. azteca was the most sensitive organism tested with LC 50 of 1.4 μg/1. G. pseudolimnaeus was also quite sensitive with LC 50 values of 7.2 and 9.0 μg/1. The series of A. intermedius run in river water gave an LC 50 of 11.8 μg/1, but the tap water series showed no significant mortality at 15 μg/1 for 240 hours. This discrepancy prevents drawing any positive conclusions for Asellus but indicates the need for more intensive investigation. We found no significant mortality D. dorotocephala at 15 μg/1 for 192 hr. These results are summarized in Table 1 and Fig. 1.

While H. azteca was more sensitive in terms of concentration than G. pseudolimnaeus, it had a higher ECT 50 at 10 μg/1, 5.3 hr compared to 1.4 hr (Table 2, Fig. 2).

DISCUSSION

Lesser's (1970) values for Gammarus are noticeably lower than ours. However, we used different water, a different temperature, and a different species of Gammarus. Any or all of these factors could account for the observed differences.
TABLE 1. TOXICITY OF ANTIMYCIN A (LIQUID FINTROL CONCENTRATE) TO SELECTED AQUATIC INVERTEBRATES AT 15°C.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Water</th>
<th>pH</th>
<th>96 hr LC 50 and 95% Confidence Interval (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Asellus intermedius</em></td>
<td>Bark River</td>
<td>8.35</td>
<td>11.8 (7.4-18.9) No significant mortality at 15 μg/l for 240 hours</td>
</tr>
<tr>
<td>(isopod)</td>
<td>Biotron tap</td>
<td>7.45</td>
<td></td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>Biotron tap</td>
<td>7.45</td>
<td>1.4 (0.9-2.2)</td>
</tr>
<tr>
<td>(amphipod)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gammarus pseudolimnaeus</em></td>
<td>Bark River</td>
<td>8.35</td>
<td>7.2 (5.3-9.7) 9.0 (6.6-12.4)</td>
</tr>
<tr>
<td>(amphipod)</td>
<td>Biotron tap</td>
<td>7.45</td>
<td></td>
</tr>
<tr>
<td><em>Dugesia dorotocephala</em></td>
<td>Biotron tap</td>
<td>7.45</td>
<td>No significant mortality at 15 μg/l for 192 hours No significant mortality at 15 μg/l for 192 hours</td>
</tr>
<tr>
<td>(planarian)</td>
<td>Biotron tap</td>
<td>7.45</td>
<td></td>
</tr>
</tbody>
</table>

Work with fishes has shown the toxicity of Antimycin A to decline as pH increases (Berger et al., 1969). Lesser's work indicates this to be true for *Gammarus* as well. Our data for *A. intermedius* and *G. pseudolimnaeus* show an opposite trend (Table 1), and some factor in river water may have acted synergistically with Antimycin A to cause a higher mortality.

The differences in ranking of ECT 50 and LC 50 values for *H. azteca* and *G. pseudolimnaeus* were unexpected, but similar differences in ECT 50s and LC 50s have been reported for several fish species (Berger et al., 1969).

Possible toxicity resulting from the acetone in the stock solution cannot be distinguished from Antimycin A toxicity in our experiments. No control was run, with acetone and water, since the primary purpose of our experiments was to determine whether certain invertebrates would be killed by Antimycin A as administered in the field, and field formulations used for river systems in Wisconsin are mixed with acetone.

Our studies indicate that *H. azteca* and *G. pseudolimnaeus* are susceptible to Antimycin A at levels used in fish management (10-15 μg/l). Since these fish food organisms might be slow to reinvade
waters once they have been eliminated, their restocking should be considered in fish management projects. Since these invertebrates are not readily available an alternative course might be to leave some parts of the drainage basin untreated for natural repopulation.

Due to their sensitivity and ease of handling, both *H. azteca* and *G. pseudolimnaeus* are suitable for bioassay of Antimycin A. These animals might be preferable to fish for bioassay work because of easy transportation and maintenance of enough individuals.
FIGURE 2—The time of exposure effect at 10 μg/1 of Antimycin A on Gammarus pseudolimnaeus and Hyalella azteca.

TABLE 2. TOXICITY OF ANTIMYCN A (LIQUID FINTROL CONCENTRATE) AT 10 μg/1 TO SELECTED AQUATIC INVERTEBRATES AT 15C.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Water</th>
<th>pH</th>
<th>96 hr ECT 50 and 95% Confidence Interval (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyalella azteca</em></td>
<td>Biotron tap</td>
<td>7.45</td>
<td>5.3 (2.3-12.5)</td>
</tr>
<tr>
<td>(amphipod)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gammarus pseudolimnaeus</em></td>
<td>Biotron tap</td>
<td>7.45</td>
<td>1.4 (0.9-2.1)</td>
</tr>
<tr>
<td>(amphipod)</td>
<td></td>
<td></td>
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</table>
ACKNOWLEDGMENTS

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