# MALATHION: CHRONIC EFFECTS ON ESTUARINE FISH

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Malathion, like many of the other organic phosphorus insecticides, is toxic to fish at relatively low concentrations (Pickering, Henderson, and Lemke, 1962; Weiss, 1961), but the probability that lethal concentrations will occur throughout large bodies of estuarine water is remote. The problem is more likely to be adverse effects of sublethal concentrations of these chemicals on fish and other estuarine organisms. Published information concerning the effects of long-term exposures of fish to organic phosphorus insecticides is scant. Pickering *et al.* (1962) who reported on a 30-day exposure of fathead minnows (*Pimephales promelas*) to Delnav® found that toxicity increased with length of exposure up to 15 days, but detected no further increase during an additional 15 days. Growth rates of cutthroat trout (*Salmo clarki*) were apparently not affected by periodic exposure to malathion in feed and water (U. S. Department of the Interior, 1965). We are unaware of any publications on the chronic effects of malathion on marine fish, although it is used extensively for mosquito control in or near estuarine areas. The present study indicates that spot, *Leiostomus xanthurus,* a common sciaenid fish which spends much of its early life in the inshore waters of the Atlantic and Gulf coasts, can tolerate prolonged exposure to a sublethal concentration of malathion.

MATERIALS AND METHODS. The study was conducted at the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida, between May and November 1965. Young-of-the-year spot were seined from local waters in February and acclimated in the laboratory 3 months

before the experiment was started. We put 150 juvenile fish, averaging 39 mm in standard length, in each of two wooden tanks lined with fiberglass. The tanks—30 cm. wide, 25 cm. deep, and 244 cm. long—had a capacity of approximately 180 liters. A constant flow of 396 liters per hour of unfiltered seawater passed through each of the tanks. Fish were maintained in the tanks over a period of 26 weeks. We did not attempt to control the temperature and salinity of the seawater flowing through the tanks. Average monthly water temperatures during the experiment varied from 23° to 29° C. and average monthly salinities ranged from 25 percent to 27 percent. Fish were fed daily on a mixture of ground fish and dog food.

A stock solution of malathion[1] was metered into one of the tanks; the other served as a control. The strength of the stock solution was adjusted so that the concentration of malathion in the experimental tank was 10 ppb (micrograms/liter). This concentration was selected on the basis of results of acute-toxicity experiments. Length of exposure is critical in determining a sublethal concentration; 50 ppb malathion killed juvenile spot only after 14 days of continuous exposure. Although some of the organic phosphorus insecticides hydrolyze rather rapidly in aqueous solutions, stock solutions of malathion prepared in water of pH 7.2 remain stable for a week. Weiss and Gakstatter (1964) reported a half-life of 50 days for malathion stored in water of pH 7.0.

Fish exposed to organic phosphorus insecticides show inhibition of brain cholinesterase (ChE) levels. To determine the brain-enzyme activity during this chronic exposure, we killed five fish from each tank every 2 weeks. Following excision and wet weighing of the brain, we prepared a homogenate in phosphate buffer of pH 7.2 with a tissue concentration of 3.5 mg. per ml. One ml. of this homogenate is incubated 20 minutes at 25° C. with 1 ml. of a 0.004 M solution of acetylcholine bromide, and the residual acetylcholine (ACh) determined colorimetrically (Hestrin, 1949). Previous studies at this laboratory showed that ChE activity of the brain of spot decreases as the brain weight increases, but that variations for a particular weight are generally less than 10 percent. A line of best fit based on data from 155 unexposed specimens was considered to represent 100 percent activity for the species. Consequently, the ChE level in the brain of a fish can be expressed as percentage of normal.

RESULTS. Neither group of fish exhibited symptoms of distress during the 26 weeks of the experiment, nor did the two lots differ significantly in growth or mortality. Brain ChE was significantly lower (90 percent level of probability) in the experimental group throughout the experiment, and appeared to stabilize at approximately 70 percent of normal. This degree of inhibition appears to have no adverse effects on these fish; we have experimentally reduced enzyme levels in spot and sheepshead minnows (*Cyprinodon variegatus*) to less than 50 percent for 48 hours without causing mortality.

Spot recover rapidly from exposure to sublethal concentrations of malathion. One week after termination of this experiment, the fish had regenerated near-normal levels of enzyme.

Immediately after termination of the chronic exposure, and again 1 week later, fish from the two groups were subjected to lethal concentrations of malathion. Differences in mortality were not significant. Brain ChE levels of the experimental fish that survived a 96-hour exposure to concentrations of 75 and 100 ppb were proportionately lower than those of control fish surviving the same concentrations.

Fish that survived the chronic exposure were subjected to a rapid drop in salinity to determine if their ability to adapt to this physiological stress was affected by malathion. We observed no differences

[1] Furnished through the courtesy of American Cyanamid Company (*O,O*-dimethyl phosphorodithioate of diethyl mercaptosuccinate).

in reactions between experimental and control groups when they were transferred directly from water with a salinity of 2.8 percent to water of salinity 1.5 percent. A gradual reduction to 0.9 percent caused distress behavior in both groups but no fish had died in 48 hours.

We observed no gross pathology of the gonads. The range of sizes of gonads in the experimental fish was similar to the range in unexposed fish of the same age group.

*Literature Cited*

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