Sublethal Effect of Malathion to Three Salmonid Species*

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Introduction

Malathion is one of the most widely used insecticides in the United States since the controlled use of DDT was put into effect. Malathion is used to control a variety of insect pests on rangeland, forest lands and agricultural lands. Many of these treated areas contain streams and lakes which harbor fish populations. Malathion dose levels for insect control is given as 1/2 to 3 pounds per acre (THOMSON, 1972). These large amounts of the toxicant, if released into aquatic environments, may create detrimental effects to fish.

The 96 hr TLm for malathion to four salmonid species was shown to be between 120 and 265 ppb (POST and SCHROEDER, 1971). Little or no information is available to indicate the sublethal effects of this insecticide on wild ranging fishes. However, the major effect would certainly be the reduction of acetylcholinesterase (AChE) reserves, which in turn would bring about the inability of body muscles to perform properly. Fish with reduced AChE reserves would probably be unable to maintain a normal upright position in the water resulting in uncontrolled drifting, inability to protect itself from predation, beaching or other hazard. Depleted AChE resynthesizes slowly. Some animals may require up to three months to return to normal enzyme levels in muscle and brain after serious depletion (KOELLE and GILMAN, 1949; MACKWORTH and WEBB, 1948). Fish may be unable to survive in a wild environment with long periods of reduced physical strength. This hypothesis led to the investigation of physical abilities of certain salmonids following quantitative reduction of AChE reserves produced by sublethal exposure to malathion.

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* This research was completed at Colorado State University, Department of Fishery and Wildlife Biology under contract with the Bureau of Sport Fisheries and Wildlife, Fish Pesticide Research Laboratory.

Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 3 © 1974 by Springer-Verlag New York Inc.

Materials and Methods

Three species of salmonids were used in the study. These were rainbow trout (Salmo gairdnerii), brook trout (Salvelinus fontinalis) and coho salmon (Oncorhynchus kitusch). Fish were held in three m long by 30 cm wide fiberglass troughs carrying water at a depth of 15 cm. The water was supplied from a well at a constant temperature of 16.5 C and near constant chemistry (POST and SCHROEDER, 1971). Volumes of water flowing through each trough was dependent upon toxicant dilution desired but was never less than 1.5 liters per minute. All pipe lines and valves were polyvinyl chloride or polyethylene. Water flow was controlled by use of a dilution apparatus and orific boxes (CLINE and POST, 1972).

Experimental fish were either reared in the water supply or were acclimatized to the water for 2 months before being used in experiments.

Phillips Malathion 55* (55% malathion emulsified concentrate) was added to distilled water to make working concentrations. The working concentration was metered into well water to make calculated dilutions capable of producing the desired effect on the fish.

Fish were held in the experimental troughs for 3 to 7 days before toxicant was added to the water. This allowed them time to become accustomed to the new environment and to feeding procedures.

Three groups of 120 fish of each species were exposed to three concentrations of malathion in order to obtain brain AChE levels of approximately 75%, 45% and 25% of the levels shown by nonexposed fish. An experimental control group of 120 fish of each species was held under the same conditions as those receiving toxicant (Table 1).

* American Fertilizer and Chemical Company, Subsidiary: Phillips Petroleum Company, Henderson, Colorado.

Table 1

Concentrations of Malathion in ppb Necessary to Achieve Fish Brain AChE Residues of Approximately 75%, 45% and 25% of Experimental Control Animals*

Approximate % of Control AChE	Brook Trout (ppb)	Rainbow Trout (ppb)	Coho Salmon (ppb)
75	40	55	100
45	90	112	200
25	120	175	300

* 7 to 10 days exposure depending on species.

Analysis for AChE in the brain of experimental fish was by the method of GARRY and ROUTH (1965). Three fish brains were analyzed as a composite and two such composites used for each sampling day from each of the four groups of fish of each species (one experimental control and three toxicant concentrations). Fish brain samples were taken at intervals between days 0 to 10 of the AChE depression period and days 14 to 42 of the recovery period. The sample collection times were somewhat dependent on fish species and rate of enzyme depletion.

Two groups of 20 fish from each of the three treated troughs and from the experimental control trough were subjected to stamina tunnel testing at the end of the malathion exposure period. The physical activity index was determined by the method of THOMAS et al. (1964), with modifications. The initial water velocity used in the stamina tunnel for the present study was 0.875 fps. The initial velocity used by THOMAS et al. was 0.475 fps.

Determination of physical activity index consisted essentially of finding the time necessary for 25% of the fish to be forced from the tunnel and the time necessary for 75% of the fish to be swept from the tunnel. Adding these two times together yielded the physical activity index for the group of fish being studied.

Groups of fish yielding desired brain AChE repression were allowed to recover to the approximate enzyme levels shown prior to malathion exposure. The time in days was recorded as the recovery time. The fish were again subjected to the same malathion concentrations used previously to determine if prior exposure altered

susceptibility to the toxicant. The second exposure period was accompanied by analysis for brain AChE levels.

Data were subjected to factorial analysis of variance to show the relationship between percentage of residual AChE in the fish brains and their ability to perform physical activity. Analysis of variance was also used to relate effect of physiological experience with malathion to a second exposure to the insecticide when the fish had returned to apparently normal brain AChE levels prior to the second exposure.

Results and Discussion

There was a very significant difference ($p=0.05$) in the concentrations of malathion necessary to reduce brain AChE residues to desired levels in the three species of Salmonids studied (Table 1). Coho salmon required approximately twice the concentration of malathion in water to produce similar AChE reductions as did brook trout and rainbow trout. This was also reflected in the 96 hr TLm for the three species (coho - 208; brook - 120; rainbow - 122; POST and SCHROEDER, 1971). The results of the acute toxicity tests imply that brook trout and rainbow trout would be more vulnerable to sublethal amounts of malathion than coho salmon.

The physical activity index of fishes with depleted AChE stores was most striking (Table 2). Brook trout and rainbow trout with AChE levels reduced to approximately 24 and 28 percent of their nonexposed levels showed less than one-third (29%) of the ability to perform physical work as compared to nonexposed fishes. The fact that coho salmon showed a greater ability to perform physical activity with approximately the same mean percent residual AChE as rainbow trout or brook trout cannot be explained. Possibly coho salmon do not absorb malathion as readily as the other two species. This is reflected by the requirement for higher concentrations to yield desired AChE reductions (Table 1). However, reduction of brain AChE to approximately 72, 52 or 29 percent of nonexposed coho salmon should yield similar activity indices as was found in brook trout and rainbow trout with similar AChE reduction.

Table 2

Brain AChE Reduction and Activity Index

Malathion Conc. (ppb)	Percent Residual AChE Mean	Range	Activity Index Mean*	Percent**
Brook Trout[a]				
0	100.0		29:18	100.0
40	68.4	65.2-72.1	24:31	83.7
90	51.4	50.6-52.9	16:15	55.5
120	24.3	21.0-28.9	8:35	29.3
Rainbow Trout[b]				
0	100.0		35:20	100.0
55	81.5	79.1-85.4	34:12	97.7
112	50.6	48.3-53.3	23:15	66.3
175	28.1	27.1-29.3	13:26	29.0
Coho Salmon[c]				
0	100.0		43:6	100.0
100	71.9	71.1-75.5	41:7	95.4
200	52.0	50.2-53.1	33:3	76.7
300	29.0	27.0-32.1	20:58	48.7

a. Average body weight 53g - % residual AChE obtained in 7 days exposure.

b. Average body weight 10g - % residual AChE obtained in 10 days exposure.

c. Average body weight 11g - % residual AChE obtained in 9 days exposure.

* Minute:Second

** Percent of physical activity index of exposed fish as compared to nonexposed fish of the same species.

The general correlation between mean percent residual AChE and physical activity index would be expected because residual AChE enters into all-or-none reactions along the neuron or at the neuro-muscular synapse (NACHMANSOHN and ROTHENBERG, 1945). If AChE is depleted to some degree, ability for synaptic function should also be depleted to a similar degree. However, the data indicate that approximately 20 to 30 percent of stored AChE can be lost by rainbow trout and coho salmon with a loss of less than 5% of their physical abilities (Table 2).

A relatively long period of time was required to recover brain AChE levels to near pre-exposure levels. Recovery time did vary with fish species. Brook trout recovered most rapidly. Even then, approximately 25 days were needed for fish with the most depleted brain AChE to return to levels similar to the nonexposed level. Rainbow trout required approximately 35 days and coho salmon approximately 42 days (Table 3). These figures are significant in that they point out the need for spacing malathion insecticide usage in ecosystems where this insecticide is used at intervals during a growing season. Other organic phosphate compounds which form firm bonds with AChE react similarly. If these compounds are used in conjunction with malathion, their effect must also be taken into consideration.

Table 3

Percent Brain AChE on Recovery and After the Second Exposure

Malathion Conc.(ppb)	AChE Recovery Percent*	Percent AChE Second Reduction Mean*	Range*
Brook Trout	25 day recovery		
40	95.9	67.7	65.9-74.5
90	102.4	50.2	47.8-51.9
120	93.9	23.7	23.0-27.7
Rainbow Trout	35 day recovery		
55	98.3	79.9	74.2-81.7
112	97.0	48.0	45.5-48.3
175	94.8	21.9	19.6-23.9
Coho Salmon	42 day recovery		
100	105.1	72.9	71.4-74.9
200	103.5	53.5	51.4-55.6
300	104.6	27.2	26.1-22.9

* Calculated from initial AChE values for that group of fish on the initial day of the experiment.

There was no statistical difference in susceptibility to a second exposure of these three species of fishes to malathion with but one exception, rainbow trout subjected to malathion concentration of 175 ppb yielded a slight and unexplained significant difference between the first and second brain AChE reduction

(p=0.05) (Table 4). These data imply that fish could be expected to react to malathion similarly at each exposure if they were allowed to recover storage of brain AChE to levels near the levels they had prior to initial exposure.

Table 4

Relationship Between Prior Exposure to Malathion and Subsequent Susceptibility

	Malathion Conc. (ppb)	Mean Brain AChE (percent) First Reduction	Second Reduction
Brook Trout	40	68.4	67.7
	90	51.4	50.2
	120	24.3	23.7
Rainbow Trout	55	81.5	79.9
	112	50.6	48.0
	175	28.1*	21.9*
Coho Salmon	100	71.9	72.9
	200	52.0	53.5
	300	29.0	27.2

* Measurable significant difference between first and second reduction (p=0.05).

The results of this study indicate that releasing malathion into lakes and streams may not produce immediate loss of fishes but may result in an inability for them to sustain physical activity in the search for food, maintain position in flowing water or in eluding predatory animals. Thus, fish may be lost as an indirect result of sublethal toxicity of the insecticide. There remains a need to correlate the laboratory results reported here with reduced physical ability and survival of wild ranging fishes. This could be accomplished by stocking fish into natural streams which had been treated with malathion to reduce AChE levels to that used in the present study. Population estimates of the stocked fish over time should indicate survival at various levels of AChE depletion. Also, the information obtained in the present study points out the need for careful planning of each insecticidal operation using malathion so that consideration be given to the physiological and biochemical alteration which may come about in animals inadvertently placed in contact with the toxicant.

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