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Survival and Condition of Rainbow Trout (*Salmo gairdneri*) After Acute Exposures to Methyl Parathion, Triphenyl Phosphate, and DEF

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Acute toxicity testing continues to play an essential role in assessing the relative toxicity of chemical pollutants to aquatic organisms. However, the methods now employed (COMMITTEE ON METHODS FOR TOXICITY TESTING WITH AQUATIC ORGANISMS 1975; ASTM 1980) do not stress evaluation of the condition of organisms that survive an acute exposure. The condition of the surviving organisms affects the rate at which an aquatic resource will recover following catastrophic chemical spills, accidental aerial spraying, or runoff from agricultural lands.

We attempted to determine long range effects resulting from acute exposure of rainbow trout (Salmo gairdneri) to three organophosphorus compounds. This toxicity test was basically a two-step process in which fish were subjected to a 96-h static acute exposure, followed by a 30-day observation period in which the survivors were held in fresh flowing water. Survival, growth, and physiological alterations were monitored to determine additional effects of the toxicant not normally measured in an acute toxicity test.

The chemicals used in this study were DEF (S,S,S-tributyl-phosphorotrithioate), an aliphatic organophosphate defoliant; methyl parathion (O,O-dimethyl O-p-nitrophenyl phosphorothioate), an aliphatic/aromatic organophosphate insecticide; and triphenyl phosphate (O,O,O-triphenylphosphate), an aromatic organophosphate that is combined with various phosphate esters in mixtures to form hydraulic fluids. This study was conducted to provide both toxicity data on these compounds and to evaluate the effectiveness of the test procedure. It is hoped that this protocol for examination of post exposure effects will provide a basis for development of methods aimed at better assessment of the effects of chemical spills on aquatic resources.

MATERIALS AND METHODS

General

Technical grade DEF (95.4%) was provided by the Chemagro Agricultural Division of Mobay Chemical Corp. and technical grade methyl parathion (76.8%) by Monsanto Chemical Co. Triphenyl phosphate (99%) was purchased from Eastman Chemical Products, Inc.

Eggs of rainbow trout were obtained from the White Sulphur Springs (West Virginia) National Fish Hatchery and hatched at the Columbia National Fisheries Research Laboratory. The tests were begun when fry were 12 days past the swim-up stage, a sensitive life stage for toxicity testing. Fish holding and acclimation procedures, temperature requirements and preparation of dilution water were performed in accordance with standardized acute toxicity methods (COMMITTEE ON METHODS FOR TOXICITY TESTING WITH AQUATIC ORGANISMS 1975).

Acute Exposures

We exposed 100 fish (avg. weight 0.11 g; avg. total length 24 mm) under static water conditions for 96 h to each toxicant in 30-liter stainless steel tanks. The acute exposure concentrations were selected to approximate 96-h EC 31, EC50, and EC69 values. The EC31 and EC69 represent a 0.5 standard deviation from the EC50, and usually elicit a significant acute response. These concentrations were determined by calculating mean values from five previous standard acute toxicity tests conducted at the Columbia National Fisheries Research Laboratory with rainbow trout of the same age. The theoretical EC values represent the cumulative effects of mortality, immobility, and loss of equilibrium of fish at a particular toxicant concentration.

Standard static acute tests in 3-liter jars with 10 fish per jar were conducted simultaneously so that a 96-h EC50 and LC50 could be determined for each of the compounds tested with this particular strain of fish. During the 96-h treatment, fish were observed daily for mortality and other overt responses.

Observation Period

After the 96-h exposure, a maximum of 40 survivors per exposure were removed from the treatment tanks, stocked in 45-liter stainless steel tanks and reacclimated to clean well water (pH 7.5, and alkalinity 237 mg/L and hardness 272 mg/L as $CaCO_3$). The flow of well water to each tank was 400 mL/min. Temperature was controlled by placing the stainless steel tanks in a waterbath (448 x 91 x 41 cm) maintained at 12 $\pm$ 1 C by Honeywell Dialatrol* temperature controllers.

Fish were fed Rangen's Salmon Starter and brine shrimp nauplii *ad libitum*, three times daily during the 30 day observation period. At day 7, the number of fish were reduced to 30 per tank to prevent overcrowding and to compensate for the potential effects of early mortality on the growth rates of survivors. At 0, 7, and 15 days after the acute exposure, fish were removed from the tanks and photographed for growth determination (MARTIN 1967). After 30 days, all fish were removed from tanks, weighed, measured (total length), and examined for physical abnormalities.

*Use of trade names does not imply government endorsement of commercial products.

We subjected growth data to one-way analysis of variance and compared means by using the least significant difference test. Statistical analysis of mortality data was performed by using the binominal chi-square analysis (SNEDECOR AND COCHRAN 1967). The EC50's and LC50's and their 95% confidence limits were calculated by the method of LITCHFIELD AND WILCOXON (1949).

RESULTS

Acute Toxicity

The 96-h LC50's (95% confidence intervals in parentheses) were 0.36 (0.31 - 0.41) mg/L for triphenyl phosphate; 0.31 (0.24 - 0.40) mg/L for DEF; and 2.8 (2.2 - 3.5) mg/L for methyl parathion. As a basis of comparison, EC values and LC values, representing a 0.5 standard deviation from the EC50 and LC50 were also determined (Table 1).

Table 1. Standard acute toxicity [96-h EC and LC values (mg/L)] of three chemicals to rainbow trout.

Chemical	EC31	EC50	EC69	LC31	LC50	LC69
Methyl parathion	1.8	2.0 (1.7-2.3)*	2.3	2.3	2.8 (2.2-3.5)*	3.3
Triphenyl phosphate	0.24	0.30 (0.24-0.37)*	0.38	0.32	0.36 (0.31-0.41)*	0.41
DEF	-	-	-	0.23	0.31 (0.24-0.40)*	0.41

*(95% confidence intervals in parentheses)

Results obtained after 96-h of static exposure in 30-L stainless steel tanks indicated that methyl parathion at concentrations of 2.1, 2.5, and 2.8 mg/L produced the most deleterious effects to rainbow trout, causing mortalities of 37%, 38% and 61%, respectively (Table 2). All survivors in each concentration were immobile and had distended abdomens at the conclusion of the static exposure.

Table 2. Chemical treatments and cumulative mortality of rainbow trout at the conclusion of 96-h static acute tests and during the post-exposure period.

Chemical and concentration (mg/L)	96-h acute exposure		Post exposure period (days)		
			1	7	7
	Immobility among survivors (%)	Mortality (%)	Mortality (%)	Mortality (%)	Recovery of immobile fish (%)
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
Methyl parathion					
2.1	100	37	72**	72**	45
2.5	100	38	83**	83**	27
2.8	100	61	98**	98**	5
Triphenyl phosphate					
0.21	4	0	0	2.5	98
0.24	7	5	5	5	100
0.29	34	6	6	6	100
DEF					
0.24	11	5	7	15*	50
0.29	20	30	37**	46**	35
0.35	24	34	44**	51**	29

*Significant ($p < 0.05$)
**Significant ($p < 0.01$)

Mortality and immobility among survivors after 96 of exposure to these organophosphates in the 30-L stainless steel tanks closely correlates with the concentration response of rainbow trout observed in the standard acute toxicity test.

Observation Period

Ability of survivors to recover after exposure to methyl parathion was significantly impaired ($p<0.01$) during the first day after their transfer to clean water (Table 2). After this critical period, no further mortality was observed. Survival after exposure was 28% for fish exposed to 2.1 mg/L of methyl parathion, but only 2% for fish exposed to 2.8 mg/L.

No significant increase in mortality of rainbow trout was observed during the observation period following exposure to triphenyl phosphate. By day 2 of the observation period, survivors that had been immobilized by this toxicant regained their locomotor activity. Another biological effect observed in about 12% of the survivors exposed to the highest concentration of triphenyl phosphate was possible vertebral damage in the caudal area, which resulted in spinal curvatures. MCCANN and JASPER (1972) observed a similar reaction in bluegills (Lepomis macrochirus) treated with several pesticides under acute conditions.

Significant ($p<0.05$) mortality of fish exposed to all DEF concentrations occurred during the first 7 days of the observation period (Table 2). The incidence of recuperation among survivors immobilized by acute DEF exposure ranged from 35 to 50% and was inversely related to toxicant concentration.

Growth of rainbow trout, expressed as mean total length, during the observation period was not significantly affected by prior acute exposure to methyl parathion, triphenyl phosphate or DEF as compared with control animals (Table 3). Mortality caused by these compounds was not selective for any particular size of fish in the population tested based on examination of pre- and post treatment growth data.

Table 3. Mean total length in mm (± SD in parentheses) of rainbow trout at day 0 and day 30 of observation period.

Chemical and acute exposure concentration (mg/L)	Days post exposure 0	30
Control	26 (2.1)	47 (4.2)
Solvent control	26 (2.2)	47 (3.3)
Methyl parathion		
2.1	26 (1.3)	50 (2.8)
2.5	25 (2.0)	49 (3.8)
2.8	26 (1.8)	51 (5.7)
Triphenyl phosphate		
0.21	25 (1.8)	48 (4.1)
0.24	25 (2.4)	48 (5.1)
0.29	26 (1.8)	47 (4.3)
DEF		
0.24	26 (2.2)	49 (3.3)
0.29	26 (1.8)	47 (4.3)
0.35	26 (1.7)	48 (4.0)

DISCUSSION

The toxicity of most organophosphate pesticides, such as methyl parathion and DEF, is partly a function of the extent to which acetylcholinesterase (AChE) inhibition occurs (O'BRIEN 1967) and the ability of the organism to reverse this inhibition or compensate for it. Although the rate of inhibition and recovery may differ in different species of fish, in general fish are characterized by a slow recovery of AChE activity (WEISS, 1961; BENKE AND MURPHY, 1974). Seemingly as a result of this factor, mortality of rainbow trout continued after their removal from acute exposure to methyl parathion and DEF. Stabilization of the fish population does not occur until susceptible individuals either succumb or presumably reverse AChE inhibition. The acute toxicity of methyl parathion to rainbow trout in this study corresponded to that observed by MACEK AND MCALLISTER (1970); however, this toxicity represents only a partial indication of the susceptibility of rainbow trout to methyl parathion since significant additional mortality occurred after the 96-h exposure.

Earlier estimates of the 96-h LC50 for rainbow trout exposed to triphenyl phosphate (MAYER et al. 1981) were also verified by our acute toxicity test. Our study demonstrated the differential ability of rainbow trout to recover from adverse effects due to acute exposure to triphenyl phosphate as compared with exposure to either methyl parathion or DEF. Although there is a scarcity of information about the toxicological mode of action of triphenyl phosphate, two possible explanations are that either the phenyl groups of triphenyl phosphate do not allow formation of a stable phosphoryl-enzyme complex at the active site of AChE or the electronic configuration of triphenyl phosphate is not conducive to producing the chemical reaction of phosphorylation that results in covalent bond formation between enzyme and inhibitor. Thus, rapid recovery of fish exposed to triphenyl phosphate indicates that the mechanism of action of this chemical under acute exposure conditions may differ from that of methyl parathion or of DEF.

This protocol should provide better information in order to predict the ability of fish to recover from acute chemical exposure.

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