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Effects of Cholinesterases of Rainbow Trout Exposed to Acephate and Methamidophos

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Acephate (O,S-dimethyl acetylphosphoramidothioate), marketed as Orthene®, is an organophosphate insecticide used extensively to suppress populations of forest insect pests (Shea & Nigam 1984). Like all organophosphate insecticides acephate's mode of action is inhibition of cholinesterase. When aerially applied acephate can contaminate rivers and streams and possibly impact aquatic insects or fish.

Brain cholinesterase (ChE) inhibition has been found in birds after application of acephate (Zinkl et al. 1979, 1980, 1984). General criteria have been established for using brain ChE activity to diagnose anticholinesterase insecticide poisoning in birds (Hill and Fleming 1982). However, similar criteria have not been determined in fish. This study was designed to determine the magnitude of brain ChE depression associated with various concentrations of acephate, the time required for brain ChE to return to normal after acephate induced depression, and the tissue concentrations of acephate associated with the above parameters. Similar information was obtained for fish exposed to methamidophos (phosphoramidothioic acid, O,S-dimethyl ester) because of the possibility for acephate to be deacylated and form methamidophos (Bull 1979).

MATERIALS AND METHODS

Rainbow trout that weighed 30-80 g and were 14-21 cm in length were obtained from the American River Trout Hatchery, California Department of Fish and Game, Rancho Cordova, California. They were maintained in a holding tank at 13°C and fed a commercial trout food twice each day at a rate of about 1% body weight per day. Trout were randomly assigned to treatment groups. The trout were allowed to acclimatize in glass aquaria for 48 h prior to testing. They were not fed for 24 h before an experiment or during exposure to the insecticide.

Acephate and methamidophos were dissolved in 30-L glass aquaria. Fish were exposed to the insecticide for 24 h. The water in the

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aquaria was maintained at 13°C by partially immersing the aquaria in flowing 13°C water. A 24-h LC_{50} for acephate was not established because the highest concentration (6400 mg/L) used in preliminary studies did not cause deaths. The subsequent cholinesterase studies were conducted using 100 or 400 mg acephate/L or 25 mg methamidophos/L. These concentrations were chosen because they are levels that might occur in heavily contaminated waters.

Brain ChE activity was determined by the Ellman et al. (1961) technique as modified by Zinkl et al. (1977). Activities were determined in duplicate at 15°C using a 1 to 20 brain homogenate in 0.1 M Tris buffer, pH 8.0. Activities are reported as milliunits (mU)/mg where one unit is defined as the conversion of 1 mole of substrate to product(s) in one m under the assay condition.

Tissue concentrations of acephate were determined by gas-liquid chromatography after extraction and clean up. Acephate was extracted in dichloromethane with a Polytron tissue homogenizer. Samples were cleaned using a combination of silica gel column chromatography and freeze-out columns. The samples were concentrated using a rotary evaporator. Acephate residues were quantified using a Perkin-Elmer 3920B gas-liquid chromatograph equipped with a 3' glass column containing 0.71% reoplex on Gas Chrom Q support, an alkali flame detector and a Hewlett-Packard 3390A integrator. Tissues were spiked with various amounts of acephate or methamidophos and run through the complete procedure to determine the efficiency of the method. Recovery of the added compounds was greater than 90%. The limits of detection were 0.05 µg/g for acephate and 0.005 µg/g for methamidophos.

Statistical analysis was performed using one way analysis of variance. When the F test indicated that significant differences between groups existed, Scheffe's test was used to determine if control trout had higher brain ChE activities than insecticide exposed trout (Kleinbaum and Kupper 1977).

RESULTS AND DISCUSSION

Brain ChE activity was depressed (38.2%) in trout exposed for 24 h to 400 mg acephate/L, but ChE was not significantly depressed (20.5%) in trout exposed to 100 mg/L (Table 1). After 24 h of being in uncontaminated water, brain ChE was still depressed (42.5%) in the 400 mg/L trout. Plasma ChE was depressed in both the 100 mg/L and 400 mg/L trout (55.9%; 66.9) immediately after the 24 h exposure and after being in uncontaminated water for 24 h (49.1%; 74.4%) (Table 2). Liver and brain concentrations of acephate were higher in the trout killed immediately after exposure than in those killed after 24 h in uncontaminated water except in the brains of the trout exposed to 400 mg acephate/L (Table 3).

Brain ChE depression for 15 days was found in trout exposed to 400 mg acephate/L for 24 h and then placed in clean water (Figure 1).

Table 1. Brain cholinesterase (ChE) activity of fish exposed for 24 h to 100 or 400 mg acephate/L

Acephate Concentration (mg/L)	Recovery Time (h)	Number	Activity (mU/mg)	Standard Deviation	Depression[a] (%)	Probability[b]
0	0	4	6.38	1.33	---	---
0	24	4	6.49	0.69	---	---
100	0	4	5.12	0 70	20.5	NS[c]
100	24	4	6.18	0.51	4.0	NS
400	0	4	3.98	1.38	38.2	0.05
400	24	4	3.70	0.87	42.5	0.05

[a]Mean of both control groups was used ($\bar{X}$ = 6.44).
[b]Probability that the mean ChE activity of the group differed from the mean ChE activity of the combined control groups.
[c]NS = Not significant.

Table 2. Plasma cholinesterase (ChE) activity of fish exposed for 24 h to 100 and 400 mg acephate/L

Acephate Concentration (mg/L)	Recovery Time (h)	Number	Activity (mU/ml)	Standard Deviation	Depression[a] (%)	Probability[b]
0	0	4	98.0	21.2	---	---
0	24	4	133.1	14.6	---	---
100	0	4	51.0	34.7	55.9	0.05
100	24	4	58.8	20.9	49.1	0.05
400	0	4	38.3	19.7	66.9	0.01
400	24	3	29.6	5.9	74.4	0.01

[a]Mean of both control groups was used ($\bar{X}$ = 115.6).
[b]Probability that the mean ChE activity of the group differed from the mean ChE activity of the combined control groups.

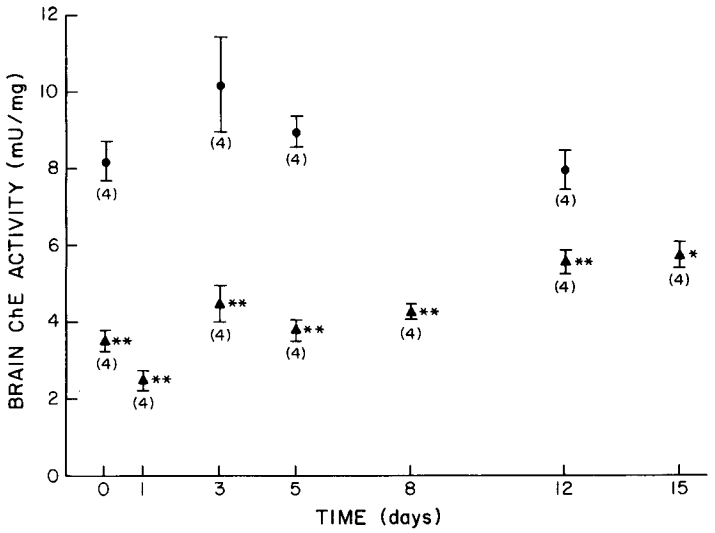

Figure 1. Brain ChE activity of trout at various times after exposure to 400 mg acephate/L. (Bar = ± 1 S.D.; ( ) = number of trout; circles = controls, triangles = acephate exposed; *Significant at $p < 0.01$; **Significant at $p < 0.001$.)

Table 3. Acephate concentrations in livers and brains of fish exposed to acephate for 24 h

Acephate Concentration (mg/L)	Recovery Time (h)	Liver Concentration[a] (µg/g)	Brain Concentration[a] (µg/g)
0	0	0.08	0
0	24	0.02	0
100	0	9.48	8.41
100	24	2.42	3.65
400	0	35.16	37.08
400	24	18.10	44.10

[a]Pooled sample of 4 livers or 4 brains.

Slow recovery was apparent during this time. Trout exposed to 25 mg methamidophos/L had significant brain ChE depression for 8 days (Figure 2). Although the brain ChE activity was less than that of the controls at 12 days, the depression was not statistically significant.

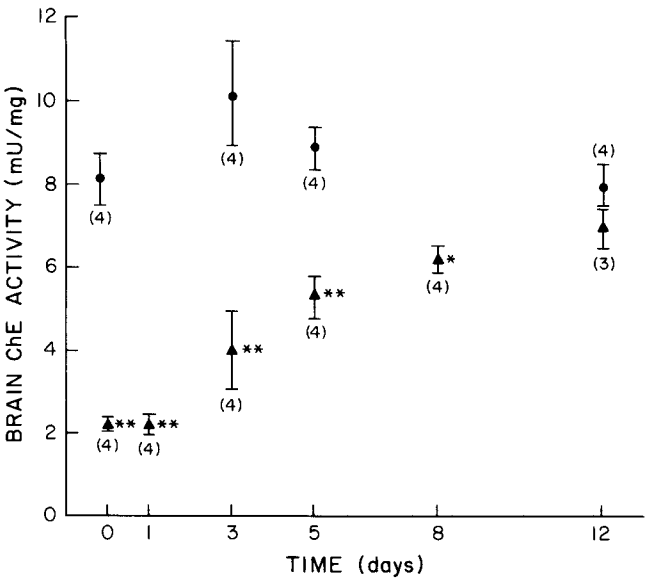

Figure 2. Brain ChE activity of trout at various times after exposure to 25 mg methamidophos/L. (Bar = ± 1 S.D.; ( ) = number of trout; circles = controls, triangles = methamidophos exposed; *Significant at $p < 0.05$; **Significant at $p < 0.001$.)

Before this study acephate was found to be of low toxicity to rainbow trout (Duangsawasdi and Kloverkamp 1979) and cutthroat trout (Salmo clarki) (Woodward and Mauck 1980). Plasma ChE activity appears to be more sensitive to acephate than brain ChE activity because the trout exposed to 100 mg acephate/L had depressed serum ChE activities but their brain ChE activities were not depressed (Tables 1 and 2).

Brain ChE activity remained depressed for 8 days after 24 h exposure to 25 mg methamidophos/L and for 15 days after a 24 h exposure to 400 mg acephate/L. These results are similar to those of another study in which 6 to 14 days were necessary for brain ChE activity to recover to normal levels in 3 species of fish exposed to the anticholinesterase cyclohexyl methylphosphonofluoridate (Weiss 1958). These results likely reflect the affinity that organophosphate insecticides have for the ChE enzymes (Matsumura 1975). Organophosphates apparently bind irreversibly to cholinesterase enzymes. Therefore, organophosphate exposed animals must synthesize new enzymes in order to return activity to normal.

Because of the low toxicity of acephate to rainbow trout, we failed to determine what levels of brain ChE inhibition suggests

that rainbow trout have died from acephate poisoning. It is apparent that the level of depression that suggests poisoning by acephate or methamidophos is greater than 70% because both insecticides inhibited brain ChE at least this much in some trout, yet no trout died. However, the persistant ChE depression (8 days for methamidophos and 15 days for acephate) suggests that sublethal effects such as an inability to sustain physical activity in the search for food, eluding predators, or maintaining position in flowing water could occur (Post and Leasure, 1974). Trout could die as an indirect result of sublethal acephate toxicity.

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