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Brain Cholinesterase Activity of Rainbow Trout Poisoned by Carbaryl

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Carbamate insecticides, especially carbaryl (N-naphthyl N-methyl-carbamate), are extensively used in forestry to suppress various forest insect pests. Over the last 10 years, millions of acres of forests have been aerially treated with Sevin^{R} (carbaryl is the active ingredient) to suppress spruce budworm (Shea and Nigam 1984). Worldwide it ranks as one of the most popular insecticides for control of agricultural pests.

Carbaryl's mode of action is that of a cholinesterase (ChE) enzyme inhibitor. Unavoidable contamination of streams and rivers during aerial application of forests present potential life threatening situations for fish. Depending upon the amount of contamination (concentration) and susceptibility of resident fish populations to the insecticide, fish may be poisoned. The determination of ChE activity has been used to diagnose carbamate and organophosphate poisoning in wild birds and to monitor the potential effects of these insecticides to birds (Hill and Fleming 1982; Zinkl et al. 1979, 1980, 1984). ChE activity has also been used for these purposes in fish (Haines 1981; Holland et al. 1967; Williams and Sova 1966), but the correlation between ChE depression and the toxic effects of anticholinesterase insecticides has not been determined. Our study was designed to determine the toxicity of carbaryl to rainbow trout (Salmo gairdneri), the magnitude of brain ChE depression that is associated with death due to carbaryl poisoning, the time required for ChE activity to return to normal after exposure to carbaryl, and the tissue concentrations of carbaryl in poisoned trout.

MATERIALS AND METHODS

Rainbow trout weighing 30-80 g and measuring 14-21 cm 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 two times each day at a rate of approximately 1% of body weight per day. Trout were randomly assigned to treatment groups. They were held for 48 h in 30-L glass aquaria before an

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experiment and were not fed for 24 h before an experiment or during the exposure to the insecticide.

The 24-h LC50 test was conducted by dissolving technical grade carbaryl in 40 ml acetone and adding the acetone-carbaryl solution to 30 L of water in the glass aquaria. The LC50 of carbaryl was determined by exposing groups of 4 trout to 0.25, 0.5, 1.0, 2.0 and 4.0 mg carbaryl/L water. Four additional trout were exposed to acetone alone. The water in the aquaria was maintained at 13^{o}C by partially immersing the aquaria in flowing, 13^{o}C water. Trout that died during the test were removed from the aquaria. If enough trout remained alive after 24 h exposure to carbaryl, 3 or 4 were killed (Table 1) for determination of brain ChE activity and brain and liver residues. The remaining trout were placed in water without carbaryl to preliminarily determine the time necessary for brain ChE activity to return to normal.

In a second experiment designed to determine the time necessary for brain ChE to return to normal, trout were exposed to 0 or 1 mg carbaryl/L for 24 h. Trout were killed immediately after the 24 h exposure. The remaining trout were placed in water without carbaryl and killed immediately and 12, 24, 48, 72 and 120 h after exposure. Brain ChE activities were determined.

Brain ChE activity was determined by the Ellman et al. (1961) technique as modified by Zinkl et al. (1977). The activities were determined in duplicate at 15oC using a 1:20 brain homogenate in 0.1 M tris buffer, pH 8.0. ChE activities were calculated in milliunits/mg (mU/mg) of brain. A unit of enzyme activity is defined as the conversion of one mole of substrate to produce(s) in one m.

A gas-liquid chromatographic (GLC) residue analysis procedure for carbaryl was adapted from the one developed by Mount and Oehme (1980) (Nakamoto and Page, 1986). Preweighed tissue samples were homogenized in acetone using a Brinkman polytron. The samples were cleaned using a combination of Florsil column chromatography and freeze-out columns. Prior to GLC analysis samples were stored in 8 mL (17 x 57 mm) scintillation vials at 0 C. Carbaryl residues were analyzed directly on a Hewlett-Packard GLC equipped with a nitrogen-phosphorus detector and a glass column (0.6 m long, 3 mm ID) packed with 2% OV-101 on Chromosorb W-HP 100/120 mesh. The limit of detection was 0.1 ng/µL of injected sample. The method was capable of determining carbaryl at 0.01 µg/g tissue.

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 groups differed from treatment groups (Kleinbaum and Kupper 1977). Probability value of <0.05 was used to determine if treatment and control groups were different. The LC50 was determined by the method of Thompson and Weil (1952).

RESULTS AND DISCUSSION

The 24-h LC50 of carbaryl to rainbow trout was 1.41 mg/L (95% confidence limits 1.02-1.95 mg/L). Signs of carbaryl toxicity included flared fins and operculums. The operculums snapped closed during breathing. Trout in the higher carbaryl concentrations had continuously flared operculums that did not close during breathing. All fish exposed to carbaryl appeared to be hyperexcitable.

In the LC50 test all fish exposed to carbaryl had depressed brain ChE activities (Table 1). The minimum depression in a trout that died was 61% found in fish exposed to 0.25 mg/L. Most fish that died had brain ChE depression greater than 85%. An apparent relationship between the concentration of carbaryl in the aquaria and the magnitude of depression was found with the fish from the highest concentrations having the greatest brain ChE depression. The fish that were placed in uncontaminated water for 24 h after exposure had brain ChE activities that were only slightly (not significant) lower than the brain ChE activities of the control fish.

Trout exposed to 1 mg carbaryl/L for 24 h and then placed in uncontaminated water for 48 h had brain ChE activities that were similar to the controls (Figure 1). Significant ChE depression was present at 0, 12 and 24 h after exposure ended.

Concentrations of carbaryl in the livers of trout exposed for 24 h to various concentrations of carbaryl ranged from 0.10 to 0.26 µg/g. In the brains the carbaryl concentrations were 0.10 to 0.63 µg/g (Table 2). The fish exposed to the higher carbaryl concentrations had the lowest brain residues. We do not have a confirmed explanation for this observation since all fish were exposed for the same amount of time. Perhaps the high dosed groups did not absorb as much carbaryl through their gills because of partial respiratory paralysis.

As other studies in fish have shown (Post and Schroeder 1971; Woodward and Mauck 1980), carbaryl is quite toxic to fish. The signs of fared fins and operculums and the snapping shut action of the operculums are probably due to the anticholinergic effects of carbaryl at the myoneural junctions associated with control of these structures. The continuously flared operculums seen in the high dose groups suggest that inability to move water across the gills (respiratory paralysis) was the cause of death. Hyperexcitability may also have been an anticholinergic effect.

The study shows that brain ChE activity can be used to diagnose poisoning of trout by carbaryl. Most trout that die from carbaryl poisoning likely will have brain ChE depression of greater than 85%, however, an occasional trout may have inhibition of only about 60%. Since brain ChE activity returns to normal within 48 h after exposure to carbaryl, monitoring of fish in field applications should be conducted soon after carbaryl application.

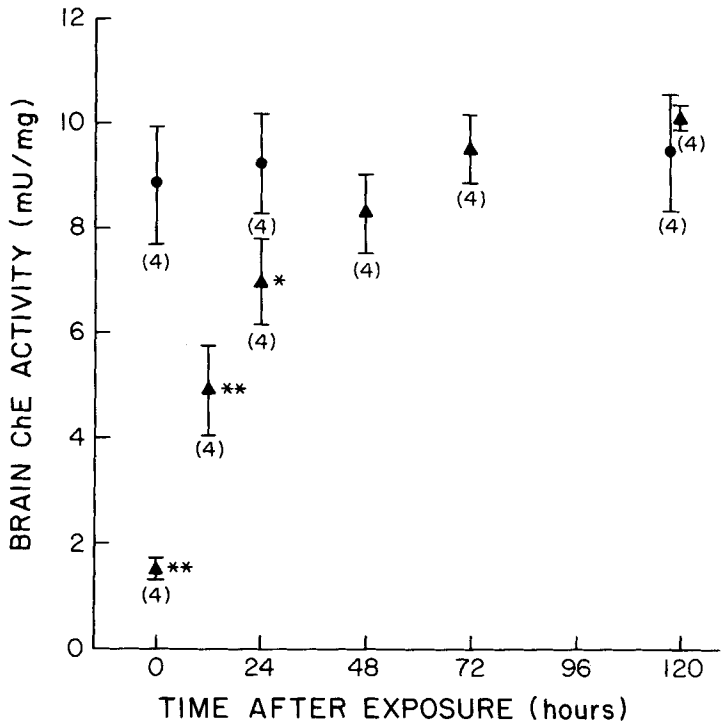

Figure 1. Brain ChE activity of trout at various times after 24 h exposure to 1 mg carbaryl/L. (Bar = ± 1 S.D.; ( ) = number of trout; circles = controls, triangles = trout exposed to 1 mg carbaryl/L for 24 h; *Significant at $P < 0.01$; **Significant at $P < 0.001$.)

If there is information indicating that trout have been recently exposed to carbaryl and their brain ChE activity is depressed greater than 60%, the diagnosis of poisoning in dead trout by carbaryl is justified. Since other carbamate and organophosphate anticholinesterase insecticides are capable of depressing brain ChE activity absolute confirmation of poisoning by carbaryl would depend upon identifying carbaryl in tissues of the fish.

Table 1. Brain cholinesterase (ChE) activity of fish exposed to various concentrations of carbaryl

Carbaryl Concentration (mg/L)	Exposure Time (h)	Recovery Time[a] (h)	Number	Activity (mU/mg)	Standard Deviation	Depression[b] (%)	Probability[c]
0	24	0	4	8.14	1.01	---	---
0	24	24	4	7.99	0.47	---	---
0.25	24	0	3	3.16	0.28	60.8	0.001
0.25	18[d]	0	1	3.18	---	60.6	---
0.25	24	24	4	7.11	0.27	11.8	NS[e]
0.5	24	0	4	1.98	0.06	75.4	0.001
0.5	24	24	4	7.69	0.93	4.6	NS
1	24	0	2	1.42	0.03	82.4	0.001
1	1.5-4.0[d]	0	2	1.14	0.21	85.9	0.001
1	24	24	4	7.02	0.38	13.0	NS
2	1.5-4.0[d]	0	4	1.03	0.26	87.2	0.001
4	0.5-4.0[d]	0	4	0.74	0.06	90.8	0.001

[a]Some of the fish were put in water containing no insecticide for 24 h.
[b]Mean of both unexposed groups was used ($\bar{X}$ = 8.06).
[c]Probability that the mean ChE activity of the group differed from the mean ChE activity of the combined control groups.
[d]Approximate time of death.
[e]NS = Not significant.

Table 2. Carbaryl concentrations in liver and brain of fish exposed to carbaryl for 24 h

Carbaryl Concentration (mg/L)	Liver Concentration[a] (µg/g)	Brain Concentration[a] (µg/g)
0	0	0
0.15	0.13	0.63
0.5	0.10	0.22
1	0.26	0.10
2	0.10	ND[b]

[a]Pooled sample of 4 livers or 4 brains.
[b]ND = Not determined.

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