Biological Effects of Fenitrothion in the Diet of Brook Trout

by
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Much of the Maritime Provinces of Canada are forested and wood products are the most important resource in the economy. Aerial insecticide spraying to control the spruce budworm, *Choristoneura fumiferana* (Clem.), a defoliating insect pest which seriously reduces tree growth, was begun in 1952 with DDT, but fenitrothion, 0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothionate, has been used since 1968. In 1972 4.6 million acres of New Brunswick were sprayed at 2 or 4 oz/acre. Numerous streams occur in the forest which are nursery areas for Atlantic salmon, *Salmo salar* L., and contain brook trout, *Salvelinus fontinalis*, important in fishing.

Previous experimental studies with fenitrothion solubilized in water show that the lethal threshold is 2.0 mg/ℓ (WILDISH et al. 1971). Sublethal effects of fenitrothion solubilized in water include inhibition of learning ability in Atlantic salmon parr (HATFIELD and JOHANSEN 1972) and increased susceptibility of parr to brook trout predation (HATFIELD and ANDERSON 1972). These effects are absent at the estimated concentration of 0.045 mg/ℓ in an average stream immediately after spraying (WILDISH et al. 1971). It has been established both experimentally (WILDISH and PHILLIPS 1972) and ecologically (ELSON et al. 1972) that aquatic insects which are the chief food resource of salmonids may be poisoned by fenitrothion solubilized in water immediately after spraying. We report here work undertaken to assess some biological effects of fenitrothion in the diet of brook trout. Analytical data on levels of fenitrothion in aquatic insects following 2 sprays both of 2 oz/acre range from 0.15 to 3.19 mg/kg (G. H. PENNEY, personal communication).

EXPERIMENTAL METHODS

Experiment 1. To determine the regurgitation threshold of fenitrothion in the diet 0.20 mls of acetone containing technical fenitrothion (98% purity) was added from a

Bulletin of Environmental Contamination & Toxicology,
Vol. 10, No. 6 © 1973 by Springer-Verlag New York Inc.

clamped micrometer syringe to the bottom half of a #3 gelatin capsule containing dry fish food. After evaporation of the acetone, capsules were fed to successive groups of 5 yearling fish per tank so that 0.78 g/fish/day was offered.

Experiment 2. Spontaneous food partitioning by individual fish was determined at 2 stocking rates (5 or 10 fish/tank). Six tanks (4 x 4 x 2', with running water) were used with fixed rations offered of 0.78 g/fish/day in low- and 0.39 g/fish/day in high-stocking rate tanks. Food was encapsulated in #3 gelatin capsules (103 mg wet weight/capsule) and daily observations with colour-coded fish made to determine individual ingested rations as described fully by WILDISH and LISTER (In preparation). Only acetone-treated (control) capsules were offered in the first 4 weeks followed by 1 mg technical fenitrothion/g food, 10 mg/g or acetone-treated food to both high- and low-stocking rate tanks. Wet fish weights were determined initially, and after 4, 6,and 8 weeks. Growth data were determined as in BRETT (1971).

Experiment 3. At the termination of Experiment 2, fish were returned to their tanks, fed the same ration as previously, and removed sequentially for dry weight and brain acetylcholinesterase activity determinations. The low density tanks from Experiment 2 containing 5 acetone-treated fish were added to the tank containing 5 fish previously fed 10 mg fenitrothion/g and food partitioning observed for a further 5 weeks (ration of 0.39 g/fish/day). Numbering of individual fish is as in Experiment 2.

Acetylcholinesterase (AChE) activity was determined titrimetrically (PICKERING and PICKERING 1971). Whole brains were removed from fish frozen at -15°C, sagittally divided, weighed (20-80 mg), portions homogenized in 2 mls of 0.7% NaCl and diluted with a further 10 mls of NaCl. Conditions were: final volume = 16 mls, substrate 0.1M acetylcholine chloride, pH = 7.5, and T = 25.0°C. Titrant was 0.02N NaOH added automatically from a 2.5-ml capacity burette. Results are means of at least duplicate determinations expressed as micromoles of liberated acid per gram wet tissue per minute (μ mol/g/min).

RESULTS AND DISCUSSION

The threshold for tolerance of fenitrothion in the stomach of brook trout was 376 mg fenitrothion/kg of

wet fish weight. Regurgitation occurred 3-24 hr after ingestion of fenitrothion and did not involve receptors in the mouth and pharynx. Regurgitation thresholds probably vary between samples of fenitrothion because the response may be caused by contaminants which differ between preparations.

Results of food partitioning by individual fish at low density suggest little change in hierarchies during control periods. Dominant fish are defined as those eating as much or more than the expected food partitioning rate, assuming equal distribution of the food available. After 4 weeks of feeding 10 mg fenitrothion/g food, the hierarchical pattern had reversed (Tables 1 and 2). No effect was observed at 1 mg fenitrothion/g food.

TABLE 1

Percentage food partitioned by individual fish per 2 weeks at a ration of 0.78 g/fish/day.

	Fish number				
Food treatment	1	2	3	4	5
Acetone	25	28	20	13	14
"	32	17	17	22	12
1 mg fenitrothion/g	29	13	19	25	14
" "	30	16	19	21	14
Acetone	26	28	24	16	6
"	25	20	23	23	9
10 mg fenitrothion/g	22	25	15	19	19
" "	14	17	18	21	30
Acetone	35	30	22	13	0
"	34	23	21	22	0
"	33	28	17	15	7
"	25	22	19	12	22

The individual ration in low and high density tanks was near that expected with equal food partitioning after 3-4 weeks of feeding with 10 mg fenitrothion/g.

TABLE 2

Percentage food partitioned by individual fish per 2 weeks at a ration of 0.39 g/fish/day.

Food treatment	Fish number 1	2	3	4	5	6	7	8	9	10
Acetone	20	17	11	14	11	16	11	0	0	0
"	15	15	16	10	15	13	16	0	0	0
1 mg fenitrothion/g	12	15	13	12	12	25	11	0	0	0
" "	12	12	13	15	12	18	17	0	0	0
Acetone	15	11	11	19	11	18	7	8	0	0
"	21	21	8	14	8	12	10	6	0	0
10 mg fenitrothion/g	9	14	14	8	8	9	5	18	13	2
" "	10	11	8	8	10	8	7	12	19	7
Acetone	22	12	16	10	12	16	12	0	0	0
"	20	19	12	14	11	4	6	14	0	0
"	12	10	12	16	10	7	8	25	0	0
"	2	17	13	15	8	14	8	23	0	0

Although growth was reduced in fish by ration reduction, food partitioned by subdominant fish previously not feeding was utilized for growth. Consequently total, but not individual, gross conversion efficiency of food per tank was highest after 4 weeks feeding with 10 mg fenitrothion/g (Table 3).

TABLE 3

Growth and gross conversion efficiency per tank for weeks 6-8 in Experiment 2.

Density treatment	Total growth change, g dry weight/fish	No. fish growing	Total gross conversion efficiency, %/fish
5/tank (1 mg fenitrothion/g	3.3	5	44.8
5/tank (10 mg fenitrothion/g	3.2	5	54.5
5/tank (Acetone	3.3	5	37.0
10/tank (1 mg fenitrothion/g	1.6	7	32.2
10/tank (10 mg fenitrothion/g	1.5	10	44.8
10/tank (Acetone	1.6	7	35.5

The results of Experiment 3 show that the previously most dominant fish in Experiment 2 (Table 4, A1 and A2) had regained dominance in the new conditions after 3 weeks. This is consistent with the view that the fenitrothion-caused changes are not persistent.

TABLE 4

Mean percentage food partitioning by individual fish per week at a ration of 0.39 g/fish/day, and density of 10 per tank. Previously fed with (A) 10 mg fenitrothion/g, (B) acetone.

	A. Fish number					
Week	1	2	3	4	5	Total
9	1	1	0	2	0	4
10	6	3	0	1	2	12
11	9	4	0	2	1	16
12	14	13	0	4	1	32
13	11	22	0	5	0	38
	B. Fish number					
9	23	39	0	16	17	96
10	25	26	1	16	20	88
11	22	21	0	22	19	84
12	13	27	0	15	13	68
13	14	17	0	16	15	62

AChE activity values demonstrate that enzyme activities had increased to 56% from 17% that of controls 27 days after exposure to 10 mg fenitrothion/g food (Table 5). At 1 mg fenitrothion/g food the depression of AChE activity (38% that of controls) was not associated with behavioural effects. AChE activities of 70% less than controls are probably necessary to cause the behavioural changes described.

TABLE 5

AChE activities of brook trout brain as μ moles acid/g/min at pH = 7.5, T = 25°C, and time from termination of fenitrothion in the diet.

Treatment	Fish Number	Sex	Time (days)	AChE Activity
1 mg fenitrothion/g	2	♂	2	0.43
	4	♀	2	0.41
	4	♀	2	0.81
	8	♂	2	0.47
	9	?	12	1.07
	10	♂	12	1.13
	6	?	27	0.81
	5	♀	27	0.58
10 mg fenitrothion/g	6	♀	2	0.21
	8	?	2	0.20
	4	♀	12	0.51
	3	♀	12	0.61
	10	♀	27	0.63
	7	♂	27	0.66
Acetone only	1	♀	2	1.12
	5	♀	2	1.16
	10	♂	2	1.14
	2	♀	12	1.37
	9	♀	12	1.24
	8	♂	27	0.84

CONCLUSIONS

The possibility that aquatic and terrestrial insects, killed by operational spray doses of fenitrothion, may cause lethal or sublethal effects directly in salmonids, has been shown by these experiments to be unlikely. The highest level found in poisoned insects by PENNEY (personal communication) is 3,000 times less than the level at which behavioural effects were noted in Experiment 2. It remains possible that reductions in insect biomass, following spraying, may be of sufficient magnitude and persistence to significantly reduce ration and hence reduce salmonid production. This possibility can only be assessed by direct field observations.

ACKNOWLEDGMENTS

We thank Mr. P.M.K. Choi for treating the contaminated food and Mrs. Madelyn Irwin for typing the manuscript.

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