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Distribution of Fenitrothion Residues in Brook Trout (*Salvelinus fontinalis*) and Lake Trout (*Salvelinus namaycush*) Tissues Following Aerial Applications to Lac Ste-Anne, Québec

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Fenitrothion (0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate) is a broad spectrum organophosphorus insecticide which has been used extensively to control spruce budworm *(Choristoneura fumiferana)* populations in eastern Canada. Although the normal procedure during aerial spraying of infested forest areas requires that the application be cut off over bodies of water large enough to be avoided, fenitrothion can still occur in streams (Eidt and Sundaram 1975) and lakes (Kingsbury 1973) in unsprayed areas because the spray may drift long distances. A number of investigators have shown that fenitrothion residues may be picked up from stream water by fish (Hatfield and Riche 1970; Coté and Tetreault 1973; Lockhart et al. 1973; Lockhart et al. 1977; Marancik 1976). There is much less information on the uptake of fenitrothion by fish in lakes (Kingsbury 1977; Kingsbury 1978). The purpose of this study was to investigate the accumulation and partitioning of fenitrothion residues among different tissues and organs in wild trout following an operational application of this insecticide to a lake.

METHODS AND MATERIALS

Lac Ste-Anne is a 142 ha oligotrophic lake located at an elevation of 366 m in the Chic Choc Mountains of the Gaspé Peninsula, Québec (Laperle 1964). In 1977, Lac Ste-Anne was within spray block 305 of the Québec spruce budworm control program. This block received two applications of fenitrothion, the first on the morning of 20 May and the second on the evening of 29 May, followed by a single application of aminocarb. The fenitrothion was applied as an oil formulation (26.3% Sumithion® Liquid Concentrate + 30.9% Aerotex + 13.4% #2 fuel oil + 29.4% #4 fuel oil) at a dosage of 280 g active ingredient/ha in a total volume of 0.84 L/ha by Douglas DC-6B aircraft equipped with boom and open-nozzle spray emission systems. Because Lac Ste-Anne was designated as an experimental spray area,

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the application was not cut off over the lake as would normally be done in an operational spray program.

Lac Ste-Anne is divided into three distinct basins separated by relatively shallow narrows. Between 7 and 24 May the middle and south basins were largely covered by ice and sampling was confined to the small, shallow (average depth 5-6 m) north basin. After 25 May the entire surface of the lake was ice-free and sampling operations were moved to the much larger and deeper (average depth 15 m) south basin for the second application.

One litre samples of water were collected at depths of 1, 2, 4, and 8 m, as well as at the surface and the bottom of the lake, using a Kemmerer type sampler. The sequence of sampling was as follows: prespray, 1, 7, 25, 49 and 97 h postspray. Samples were held at 4°C in glass bottles until extracted.

Gangs of gill nets consisting of 30 m sections of 5.1, 7.6 and 10.2 cm mesh were used to collect brook trout *(Salvelinus fontinalis)* and lake trout *(Salvelinus namaycush)*. Each gang was run out 90 m from a point of attachment on shore and anchored on the bottom. Fish were weighed, measured and dissected within 2 hours of removal from the nets. The liver, ovaries if present, intestine, any fat in the abdominal cavity and a filet of muscle with skin attached from the back between the operculum and dorsal fin were removed from each lake trout, wrapped separately in aluminum foil and frozen immediately in a chest-type home freezer. In most cases tissue samples from more than one fish were pooled to make a composite sample. Because of their smaller size, all but one of the brook trout were frozen whole.

Within 2 hours of collection each water sample was transferred to a 2 litre separatory funnel containing 100 g of sodium sulfate. The sample bottle was subsequently rinsed with 50 mL of hexane and the rinse product added to the decanter. The contents were agitated for 2 minutes and then allowed to separate and the hexane phase was collected in a 500 mL round bottom flask. The extraction was repeated twice more with 25 mL of hexane. The hexane extract was dried on 100 g of sodium sulfate and then concentrated to 1 mL with the aid of a rotary evaporator in a water bath at 38°C.

After thawing each tissue sample was cut into small pieces and homogenized with a food chopper. A 20 g portion of the homogenate was put in a Waring Blender with 10 g of sodium sulfate and 100 mL of ethyl acetate and the contents were mixed for 3 minutes. The organic extract was decanted on glass-wool and collected in a 500 mL round bottom flask. The extraction was repeated twice more with 75 mL of ethyl acetate. The 3 extracts were combined and dried by filtration under vacuum by passing through 100 g of sodium sulfate in a buchner funnel. The filtrate was concentrated to 20 mL under reduced pressure with the aid of a rotary evaporator in a water bath at 38°C.

A 10 mL aliquot of the extract was set apart and diluted in 50 mL of acetonitrile. The mixture was placed in a separatory funnel and a partition was effected with two portions of 25 mL of hexane. The polar phase of acetonitrile was conserved and concentrated to approximately 5 mL. The concentrated extract was purified by chromatography on a column (30 cm x 22 mm inside diameter), equipped with a fritted glass funnel and a vacuum tube, containing 2 cm of sodium sulfate, 10 g of an activated charcoal-celite (6:4) mixture and 2 cm of sodium sulfate. The column was first washed with 100 mL of a benzene-ethyl acetate (4:1) mixture. The column was eluted with 200 mL of the same mixture and the eluate was then concentrated to 5 mL.

A Varian 2100 gas chromatograph equipped with a flame ionization detector fitted with a rubidium head and a glass column (180 cm x 4 mm inside diameter) packed with 4% OV-101 + 6% OV-210 + 3% OV-17 on Gas Chrom Q (80-100) mesh was used for the analysis of fenitrothion. The temperatures of the injector, the oven and the detector were 230, 200 and 240°C respectively. The flows of nitrogen, hydrogen and air were 20, 35 and 200 mL/minute respectively.

RESULTS AND DISCUSSION

Fenitrothion residues rapidly dispersed throughout the lake waters following treatment (Table 1). One hour after the first application fenitrothion residues in the north basin were concentrated near the surface (0.80-0.90 µg/L), but had already penetrated to the bottom in small amounts (0.06 µg/L). Six hours later residues were fairly evenly distributed throughout the water column (0.91-1.49 µg/L). Residue levels were similar at all depths and still quite high (0.41-0.46 µg/L) 97 hours after treatment, but declined rapidly in the next 48 hours (ND-0.06 µg/L).

Fenitrothion concentrations in the surface waters of the south basin 1 hour after the second application were very high (3.01-51.65 µg/L). Residues also dispersed rapidly throughout the water column following this treatment, but appeared to decline more quickly than after the first application reaching nondetectable levels by 97 hours postspray.

A total of 27 lake trout (8♂ and 19 ♀) and 12 brook trout (2 ♂ and 10 ♀) were collected for insecticide residue analysis between 7 May and 6 June. Lake trout averaged 1585.3 g in weight (range 202.2-2872.1 g) and 56.8 cm in total length (range 29.4-68.0 cm); brook trout averaged 235.1 g in weight (range 99.9-582.2 g) and 27.3 cm in total length (range 22.4-37.5 cm).

Table 1. Fenitrothion residues in water (µg/L) from Lac Ste-Anne following aerial applications to control spruce budworm populations in the Gaspé region of Québec in 1977+.

First Application (North Basin)						
Depth (m)	0	1	2	4	8	Bottom
20 May (H1)	0.90	0.80	T*	T	0.09	0.06
20 May (H7)	1.05	0.91	0.97	1.00	1.49	1.19
21 May (H25)	0.82	0.75	0.82	1.00	1.02	0.83
22 May (H49)	ND**	0.01	0.30	0.20	0.64	-
24 May (H97)	0.41	0.43	0.46	0.42	0.42	0.44
26 May	ND	T	0.06	-	-	ND

Second Application (South Basin)						
Depth (m)	0	1	2	4	8	Bottom
26 May	ND	-	-	-	-	-
29 May (H1)	51.65	3.87	3.01	0.67	0.53	-
30 May (H7)	2.66	0.49	1.43	0.16	ND	0.12
30 May (H25)	0.01	0.42	0.62	0.55	0.58	ND
31 May (H49)	1.27	1.32	1.22	ND	0.28	0.61
2 June (H97)	ND	ND	ND	ND	ND	ND

+ Fenitrothion was applied to the lake at a dosage rate of 280 g AI/ha in a total volume of 0.84 L/ha on the morning of 20 May 1977 and again on the evening of 29 May 1977.

* T = Trace

** ND = Nondetectable (limit of detection 0.01 µg/L).

All postspray tissue samples contained measurable levels of fenitrothion (Table 2). Fenitrothion residues in the fat, muscle, intestine and ovaries of lake trout gradually increased to peak levels (280.0, 96.8, 96.3 and 48.2 ng/g respectively) 4 daysafter the first application (24 May). Residues in the liver peaked (16.1 ng/g) 2 days after the first application (22 May), but were still almost as high (14.8 ng/g) 4 days after treatment (24 May). Much higher levels of fenitrothion were measured in the fat, muscle, intestine and liver (664.0, 306.0, 313.0 and 96.6 ng/g respectively) 1 day after the second application (30 May). Residue levels in these tissues tended to fluctuate on subsequent sampling dates but were still high 8 days after treatment (6 June). The highest level of fenitrothion measured in any tissue was 665.0 ng/g in the visceral fat of lake trout on this date. Fenitrothion concentrations in lake trout tissues were generally highest in the visceral fat and lower in the muscle, intestine, ovaries and liver in that order.

Table 2. Fenitrothion residues in lake trout and brook trout (ng/g) from Lac Ste. Anne following aerial applications to control spruce budworm populations in the Gaspé region of Québec in 1977[+].

	First Application (North Basin)						Second Application (South Basin)				
	Prespray	Postspray					Prespray	Postspray			
	7–12 May	20 May	21 May	22 May	23 May	24 May	26 May	30 May	31 May	1 June	6 June
No. of lake trout sampled	5	2	1	2	2	1	2	2	1	3	3
Residues Fat	ND*	12.0	34.0	151.0	138.0	280.0	18.5	664.0	283.0	470.0	665.0
Muscle	ND	41.6	56.5	74.0	66.5	96.8	11.2	306.0	121.0	123.0	133.0
Intestine	ND	5.8	15.0	34.0	24.2	96.3	2.5	313.0	116.0	44.2	114.0
Liver	ND	4.7	2.7	16.1	2.4	14.8	1.1	96.6	17.9	27.6	39.6
Ovaries	ND	4.9	13.1	–	19.4	48.2	–	–	–	–	–
No. of brook trout sampled	5	0	1	1	1	1	2	1	0	0	0
Residues Fat	–	–	–	–	–	–	–	214.0	–	–	–
Muscle	ND	–	56.7	107.0	100.0	75.5	11.7	156.0	–	–	–
Intestine	–	–	–	–	–	–	–	117.0	–	–	–
Liver	–	–	–	–	–	–	–	28.4	–	–	–
Ovaries	–	–	–	–	–	–	–	167.0	–	–	–
Viscera	ND	–	49.0	183.0	145.0	230.0	16.2	–	–	–	–

[+] Fenitrothion was applied to the lake at a dosage rate of 280 g AI/ha in a total volume of 0.84 L/ha on the morning of 20 May 1977 and again on the evening of 29 May 1977.

* ND = Nondetectable (limit of detection 0.4ng/g for lake trout; 2ng/g for brook trout).

Fenitrothion residues in the muscle of brook trout peaked (107.0 ng/g) 2 days after the first application (22 May). Residues in the viscera peaked (230.0 ng/g) 4 days after treatment (24 May). Except for the first sample collected 1 day after treatment, residue levels were higher in the viscera than in the muscle. In the one brook trout caught after the second application, fenitrothion residues were highest in the visceral fat (214.0 ng/g) and progressively lower in the ovaries (167.0 ng/g), muscle (156.0 ng/g), intestine (117.0 ng/g) and liver (28.4 ng/g).

Fenitrothion residues accumulated fairly rapidly in brook trout and lake trout tissues. Peak levels of fenitrothion were reached 2-4 days after the first application and 1 day after the second application and were many times higher than in the surrounding waters of Lac Ste-Anne (Tables 1 and 2). Similar results have been reported by a number of other workers, both in lakes (Kingsbury 1977; Kingsbury 1978) and in streams (Lockhart et al. 1973; Lockhart et al. 1977; Marancik 1976). In the laboratory, Kanazawa (1975) and Takimoto and Miyamoto (1976) have reported bioaccumulation ratios (concentration in fish/concentration in water) for whole body residues of fenitrothion in rainbow trout *(Salmo gairdneri)* and Motsugo *(Pseudorasbora parva)* ranging from 200 to 250. Although it is difficult to calculate from the field data bioaccumulation ratios comparable to those generated in controlled laboratory tests, it appears that in certain tissues fenitrothion may have been concentrated beyond these levels. As an example, the average concentrations of fenitrothion in water and lake trout fat on 24 May were 0.43 µg/L and 280.0 ng/g respectively (Tables 1 and 2), giving a bioaccumulation ratio of approximately 650. In other tissues fenitrothion residues were much less concentrated (e.g. on the same date the bioaccumulation ratio for lake trout liver tissue was approximately 34). Since muscle makes up the bulk of a fish other than the skeleton (Lagler et al. 1962), a fairly close agreement between the bioaccumulation ratios calculated for muscle tissue and whole body residues could be expected. In fact, the bioaccumulation ratio for muscle tissue on 24 May was 225, which is within the range of values reported by Kanazawa (1975) and Takimoto and Miyamoto (1976).

Fenitrothion residues continued to persist at high levels in lake trout tissues up to at least 8 days after the second application (6 June), even though residues in water had declined to non-detectable levels 4 days earlier (Tables 1 and 2). This tendency toward retention of fenitrothion residues by trout is contrary to the findings of Takimoto and Miyamoto (1976). In their laboratory study fenitrothion residues in rainbow trout and Motsugo decreased quite rapidly (to 1/1000 initial concentration in 5 days) when fish which had been previously exposed to fenitrothion were transferred to fresh water. A possible explanation of this difference is suggested by a comparison of the temperatures to which fish were exposed in the two studies. In the laboratory tests water temperatures were held constant at 18°C for rainbow trout and 23°C for

Motsugo (Takimoto and Miyamoto 1976), whereas in Lac Ste-Anne water temperatues were much colder, ranging from approximately 2 to 5°C between 9 May and 6 June (Mathieu and Provencher 1978). Adamson and Sieber (1974) have pointed out that environmental temperature is an important factor in the detoxication and metabolism of xenobiotics by fish. It is possible that the very low spring water temperatures in Lac Ste-Anne at the time of our study may have reduced the ability of lake trout to metabolize and eliminate fenitrothion residues from their tissues. Another possiblity is that lake trout continued to be exposed to fenitrothion residues, through contaminated food organisms for example, even after detectable residues had disappeared from the lake waters.

Information in the literature on the distribution of fenitrothion residues in fish tissues is scant. Takimoto and Miyamoto (1976) studied the metabolism of fenitrothion in rainbow trout by tracing radio labelled fenitrothion taken up from water under static conditions. They found that after 6 hours exposure the concentration of radioactivity was highest in the gall bladder and intestines, but that after 24 hours the radioactivity was distributed in every tissue except the brain and heart. Lockhart et al. (1973) determined tissue concentrations of fenitrothion in rainbow trout exposed to fenitrothion in a continuously flowing system. Under their experimental conditions fenitrothion concentrations in the liver, kidney and muscle were similar indicating little significant partitioning between these organs. In the Lac Ste-Anne study brook trout and lake trout accumulated roughly similar levels of fenitrothion and fenitrothion was partitioned between tissues with fat being the principle repository for the residues followed by muscle, intestine, ovaries and liver in that order. It is suggested that the observed distribution of fenitrothion may be at least partly due to differences in the fat content of these tissues. Some support for this hypothesis is provided by Seguchi and Asaka (1981) who demonstrated that the bioconcentratin of another phosphorothioate insecticide (diazinon) by fish was closely related to the fat content of the fish. A similar relation was recognized by Roberts et al. (1977) with bioaccumulation of chlordane by northern redhorse suckers.

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