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Toxicity to Aquatic Organisms of Pond Water Contaminated by Fenitrothion during Forest Spraying

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Fenitrothion (O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate) has been shown to be toxic to a wide variety of aquatic organisms (Fairchild et al. 1989; NRCC 1975; Symons 1977). Its use in forest spray programs against the spruce budworm and hemlock looper results in the contamination of water bodies. The most recent review of the aquatic environmental fate and effects of fenitrothion (Fairchild et al. 1989), speculated that small forest ponds were the water bodies at greatest risk of receiving deposits which could have substantial impacts on resident biota. A subsequent study confirmed that the concentrations of fenitrothion in small ponds within operational spray blocks were much higher than those previously measured in larger water bodies (Ernst et al. 1991). The water concentrations of fenitrothion measured in that study were within the range of concentrations known to be acutely lethal to aquatic organisms, including fish.

This study was undertaken to determine whether deposit of fenitrothion in small ponds within operational spray blocks was sufficient to be acutely lethal to rainbow trout (Oncorhynchus mykiss) and Daphnia magna.

MATERIALS AND METHODS

The sampling sites were two small ponds located within the area proposed for the 1991 New Brunswick spruce budworm spray program. Pond 1 was an unnamed pond located approximately 25 km west of Heath Steele (47°18"1' N, 66°14"17' W). The pond was approximately 75 m by 100 m with an average depth of 1 m, being 1.5 m at the deepest point. The dominant surrounding vegetation was mature black spruce (Picea mariana) approximately 15 m in height which grew to within 10 m of the pond margin. The pond was fringed by alders (Alnus rugosa) which grew to within 2 m of the pond margin.

Pond 2 was also an unnamed pond and was situated 500 m west of Pond 1 (47°18"27' N, 66°14"39' W). It was surrounded by black spruce which was approximately 15 m in height and grew to within

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10 m of the pond margin. The pond was approximately 120 m by 50 m, had an average depth of 0.75 m and was 1.5 m at the deepest location.

Approximately 1 hr prior to spraying, five deposit samplers were randomly distributed throughout each pond, within 2 m of the margin. Deposit samplers have been previously described (Ernst et al. 1991) and consist of cellulose filter papers which were 23 cm in diameter, clipped to stainless steel plates which had previously been rinsed with pesticide-grade hexane. At the time that deposit samplers were deployed, pre-spray surface samples (2) were taken from separate locations by immersing a 1-L amber glass bottle, which had previously been detergent washed and rinsed with distilled water, acetone and hexane, such that the top 5 cm of water was collected. Water samples were placed on ice in coolers.

In addition, a water sampling station was established prior to spraying which used a peristaltic pump to draw pond water through 3-mm I.D. Teflon tubes to a 20-L glass carboy which had previously been cleaned in the same way as the 1-L bottles. The intakes of the tubes were fastened to a rod which was driven into the pond bottom 2 m from the margin such that water was drawn from 0.5 cm below the pond surface. Water sampled in this manner was to be used for bioassays.

On June 16, 1992, between 0715 and 0725 hr spraying was conducted in the vicinity of both ponds by a formation of three Grumman TBM Avenger aircraft from Forest Protection Limited, Fredericton, New Brunswick. This was the second application to the spray block, the first application having taken place 11 d previously. The aircraft were equipped with Tee Jet 11010 nozzles emitting a solution which consisted of 11% fenitrothion (active ingredient), 1.5% Dowanol TPM(solvent), 1.5% Atlox 3409F (surfactant) and 86% water. The total volume of spray emitted was 1.46 L/ha for a nominal application rate of 210 g active ingredient/ha. Aircraft height was approximately 25 m above the forest canopy. A very slight breeze was blowing from the north; however, it was so light as to be undetectable with a hand-held cup anemometer. Over Pond 1, one spray aircraft in the formation appeared to briefly shut spray booms off, possibly omitting a portion of the pond from direct application. Spraying appeared to be continuous over Pond 2. A subsequent examination of Forest Protection Limited spray reports indicated that spraying was continuous over both ponds.

Approximately 15 min after spraying, filter papers were removed from the deposit samplers and placed in 500-mL glass bottles which had previously been detergent washed and rinsed with distilled water, acetone and hexane. The bottles were then filled with medical grade nitrogen (high purity 99.99%) and placed on ice in coolers.

At the same time, five surface water samples were obtained in 1-L bottles at random locations in the manner described above. At the

time that samples were taken, 100 mL of hexane were added and the sample shaken vigorously for 5 min before they were placed in coolers on ice.

Pumping to collect bioassay samples was also initiated 15 min post-spray; however, the low rate of collection required that sampling be supplemented with three surface water samples collected in 4-L glass carboys which had been previously cleaned as described above. That 20-L composite sample was designated for rainbow trout bioassays. A single 4-L sample was also obtained at that time to be used in conducting Daphnia magna bioassays. These water samples were also placed on ice.

All sampling was completed within 1 hr of initiation of sample collection. Samples were transported to the Environmental Protection Laboratory at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia, within the same day. Fish bioassays were initiated the same day that samples were collected, Daphnia bioassays and chemical analyses were initiated the following day.

Analysis of water samples for fenitrothion content was conducted according to a previously published method (Department of Environment 1979), with minor modifications. In addition to the 100 mL of hexane which was added at the time of sampling, two further extractions were completed in the laboratory using 60 mL of hexane each time. The extracts were combined, dried over anhydrous sodium sulphate and concentrated to 1 mL by rotary vacuum and nitrogen stream evaporation. Additional clean-up of the sample extracts was not required. Instument analysis was performed on an HP5700 gas chromatograph equipped with a flame photometric detector. The accuracy and precision of the analytical procedure was verified by replicate analysis of spiked (0.5 ug/L) water samples. The procedure afforded a mean recovery of 94% ± 8.0%.

Deposit filters were cut in 2 cm strips and extracted twice with 75 mL of hexane on a wrist action shaker for 30 min. The combined extracts were concentated, or diluted to appropriate volumes in hexane for instument analysis. Quality control analyses of fenitrothion on replicate spiked (130 ug) filter samples afforded a mean recovery of 86% ± 3.5%.

Bioassays were static, acute (96-hr for trout and 48-hr for Daphnia) and conducted according to standard methods (Environmental Protection 1990a, 1990b). Rainbow trout tests were conducted on 40 litres of aerated, undiluted pond water which contained 7 fish in each exposure tank. Control exposures were conducted on Dartmouth municipal water which had been passed through activated charcoal filters and ultra-violet sterilizers. The water was soft, low in heavy metal content and had a limited buffering capacity. The characteristics of that water have been previously published (Doe et al. 1988). The tests were conducted using fish with a length of 5.6 cm (range 4.5-6.2 cm) and a mean

weight of 1.7 g (range 0.7-2.0 g). The light regime for holding fish and for tests was 16 hr light, 8 hr dark. During exposures, temperature ranged from 14.0 to 15.0°C, dissolved oxygen ranged from 9.8 to 10.4 mg/L and pH ranged from 7.0 to 8.0. Daphnia tests were conducted on 100%, 50%, 25%, 12.5% and 6% dilutions of pond water as well as dilution water control. The bioassays were conducted on the soft water from Pond 1 (12 mg/L) with no attempt to increase hardness. Water from Pond 2 was also soft (12 mg/L), however, tests were conducted on that water as well as water for which hardness was increased to 27 mg/L. Dilution water was EPA reference water (Environmental Protection 1990b). There were 10 animals (≤24 hr old) in each unaerated exposure solution of 150 mL. Photoperiod was 16 hr light/8 hr dark, temperature ranged from 18.5 to 20.5 °C, dissolved oxygen ranged from 8.8 to 9.4 mg/L and pH ranged from 7.2 to 8.1. The LC 50s were determined using the method of Stephan (1977).

RESULTS AND DISCUSSION

Analysis of samples for fenitrothion content (Table 1) indicated that Pond 1 received less deposit than did Pond 2. Deposit of pesticide during applications to forests is known to be highly variable (Pierce and Ernst 1988). Previous investigations have documented deposits which range from 12 to 176 g a.i./ha on ponds directly oversprayed (Ernst et al. 1991) and 12 to 145 g a.i./ ha in open areas of forest spray blocks (Sundarum 1990).

The deposit on collectors in Pond 1, therefore, was lower than those ranges and may represent an effort at avoidance of direct deposit on the part of the spray pilots. The deposit which was measured on collectors in Pond 2 is judged to be moderate, being close to the mean of previously reported values.

Table 1. Deposit of fenitrothion on filter paper (n=5) and concentrations of fenitrothion in water (n=5) from two ponds after forestry applications.

Pond	Deposit on filters (ug/filter, $\bar{x}$ ± s.d.)*	Deposit (g/ha, $\bar{x}$ ± s.d.)**	% Deposit (Compared with emitted rate 210 g/ha)	Water concentration	
				Pre-spray (ug/L, $\bar{x}$)	Post-spray (ug/L, $\bar{x}$ ± s.d.)
1	20.8 ± 8.2	5 ± 2	2.2 ± 0.9	0.1	10.3 ± 4.6
2	400 ± 94	88 ± 21	42.1 ± 9.9	0.1	821 ± 274

* Total area of filter 416 cm^2
** Deposit = (Deposit on filters x 10^{-6}) ÷ (Area of filters x 10^8)

The fenitrothion content of surface water was comparable to the deposit on filter papers, in that Pond 2 had a much higher concentration immediately after spray than did Pond 1. Again, the mean concentration of fenitrothion in Pond 1 was less than the mean surface water concentrations previously measured in small ponds which received direct applications of fenitrothion emitted at 210 g a.i./ha with the same delivery equipment (Ernst et al. 1991). The mean concentration of fenitrothion in Pond 2 was slightly greater than those reported by Ernst et al (1991), in which mean surface concentrations measured 15 min post spray ranged from 20 to 1500 ug/L (N=10). The fenitrothion concentrations in Pond 2 were within the range of concentrations known to be acutely lethal to a variety of fish species during short term (up to 96 hr) exposures in the laboratory (Fairchild et al. 1989). They are also approximately 10 times greater than the concentrations measured in a similar manner after experimental hand applications to bog ponds, which resulted in significant ecological disruption due to extensive invertebrate mortality (Fairchild and Eidt 1993).

The results of bioassays are presented in Table 2. While no mortality of either test species was observed when they were exposed to water from Pond 1, the water from Pond 2 produced approximately 30% mortality in the exposed rainbow trout within 96 h and caused greater than 50% mortality in exposed Daphnia magna within 48 h. It is worth noting that within 96 h, all Daphnia exposed to Pond 2 water were killed, even in the lowest dilution tested - 12.5% and all were immobilized in less than 48 hr. In 100% exposures, 50% of the Daphnia were dead within 48 hr in the pond water which had been adjusted to a hardness of 27 mg/L, while there was 90% mortality in the 'natural' pond water which had a hardness of 12 mg/L. The trout which were killed during exposure were observed to have the post - mortem symptoms of fenitrothion exposure including abdomens which were distended due to the presence of a clear fluid in the abdominal and stomach cavities. These symptoms have been previously reported (Doe et al. 1988; Klaverkamp et al. 1977).

Table 2. Mortality of rainbow trout (Oncorhynchus mykiss) and Daphnia magna exposed to surface water ponds sprayed with fenitrothion.

Treatment	Rainbow Trout		Daphnia magna		LC50*
	Test Duration	Mortality	Test Duration	Mortality	
Pond 1	96 hr	0%	48 hr	0%	n/a
Control	96 hr	0%	48 hr	0%	n/a
Pond 2	96 hr	29%	48 hr	50%	76.5%
Control	96 hr	0%	48 hr	0%	n/a

* LC50 determined by probit method of Stephan 1977.

The bioassay data indicated that fenitrothion was deposited on Pond 2 in sufficient quantities to cause acute lethal effects. It is recognized that the method of sampling maximized the toxic effects of the pond water. Pesticide which is deposited on ponds is known to be concentrated on the surface (Maguire and Hale 1980). One study has demonstrated that 70% of the fenitrothion in the microlayer of ponds is lost to the atmosphere through volatilization (Maguire 1991). Other data indicates that fenitrothion mixes readily in the water column, with maximum pond water concentrations at 0.3 m and 1.0 m depths being 1/2 and 1/4, respectively, of those at the surface (Fairchild 1993). If a conservative assumption is made that the mixed water column concentration would be 1/5 the surface water concentration, Pond 2 would have had a mean water column concentration of 165 ug/L. That concentration is less than the published LC 50 values for salmonid fish which range from 0.7 - 1.9 mg/L (Doe et al. 1988; Sanders et al. 1983; Wells et al. 1979) and those concentrations would be further diminished by degradation and adsorption. It is probable, therefore, that mortality of native fish, if it occurred, would be confined to only sensitive individuals. The mixed water column concentration would be well above the 48 hr LC 50 for Daphnia magna of 10 ug/L. (Ernst and Doe 1989), and significant native invertebrate mortality would be likely.

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