Persistence of Hexazinone (Velpar), Triclopyr (Garlon), and 2,4-D in a Northern Ontario Aquatic Environment

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A field study was conducted in enclosures located in a typical bog lake in a sandy soil area near Matheson in northeastern Ontario. Three groups of six polyethylene enclosures each were constructed and installed in the lake. Three enclosures each were treated with triclopyr (butoxyethanol ester), with 2,4-D (isooctyl ester), and with hexazinone (solution) at rates equivalent to 0.3 and 3.0; 1.0 and 2.5; and 0.4 and 4.0 kg/ha, respectively. Water, sediment, and enclosure wall samples were analyzed for residues, and temperature and oxygen levels were measured. A significant dose-dependent change in oxygen concentration was only seen in the hexazinone-treated enclosures. Rates of dissipation of 2,4-D were similar at both concentrations, and within 15 days less than 5% remained in the water. Up to 25% of the 2,4-D adsorbed to the sides of the corrals. Triclopyr concentration in water was below 5% from day 15 and could not be detected from day 42 onward. The amount of pesticide adsorbed to the sides of the enclosures was lower and appeared to dissipate more rapidly than was the case with 2,4-D. At the lower application rate, hexazinone was undetectable 21 days and at the higher rate 42 days after application. Hexazinone dissipated more rapidly than 2,4-D and was not adsorbed to sediments.

The feasibility and necessity of intensive reforestation and manangement programs are perhaps the most important questions facing Canada's forest industry. Herbicide use for site preparation and for conifer release is likely to be an important tool in any intensive forest production system. Herbicides with potential for use in forestry are triclopyr (Garlon), a pyridine analogue of the phenoxy herbicides, and hexazinone (Velpar), a triazinetype herbicide. Although forestry herbicides are unlikely to be deliberately applied to water in large quantities, it is probable that some overspraying, drift, and runoff will occur and that this may cause some environmental impact. As has been pointed out (NRCC, 1978), long-term studies on the impact of the phenoxy herbicides have not been carried out under conditions that are relevant to aquatic systems in the northern parts of Canada. Earlier studies on the persistence of 2,4-D and 2,4-DP in northern vs southern agricultural and forest soils showed that these herbicides tended to persist for longer periods in the sandy soils associated with the northern forest agroecosystem (Thompson et al., 1984). Longer persistence is a possible indicator of environmental problems and is a logical first step in the assessment of environmental impact. This study was designed to address this question in the context of a northern aquatic ecosystem.

MATERIALS AND METHODS

Selection of Field Site. A number of possible lake sites were investigated in the Matheson area. The final choice was lake 2 in Thackeray Township ($48^{\circ}25''$ N:79°57'' E). The lake is about 5 ha in area, is a typical bog lake in a sandy soil area, and by SCUBA inspection was found to have a soft sediment suitable for the sealing of enclosures (limnocorrals). The lake water was found to be pH 4.5, and although the lake was devoid of fish (not having been stocked), visual observation indicated the presence of large numbers of invertebrates, particularly larval, nymphal, and adult insects. As such, the lake was ideal for the use of enclosures and was typical of lakes in the area.

A set of 18 limnocorral support frames were constructed as previously described (Solomon et al., 1980). The supports (consisting of sections $150 \times 200 \times 2000$ mm) were **Table I. Corral Treatments**

enclosure	chemical	vol used, mL	nom rate, kg/ha	nom concn, $\mu g/L$	
1, 3, 5	2,4-D	0.67	1.0	40	
2, 4, 6	2,4-D	1.67	2.5	100	
7, 9, 11	Garlon 4E	0.25	0.3	12	
8, 10, 12	Garlon 4E	2.5	3.0	120	
13, 15, 17	Velpar L	0.67	0.4	16.75	
14, 16, 18	Velpar L	6.67	4.0	167.5	

manufactured from 25×150 mm pressure-treated spruce filled with styrofoam for flotation purposes. The support frames were bolted together on shore, floated out onto the lake, and anchored in place in the lake with the aid of concrete block anchors. The average depth of water was 2.5 m. Visual inspection of the sediments under the support frames revealed the absence of any logs, rocks, etc., that could interfere with the sealing of the corrals. The enclosure walls consisted of a 6-mil (UV-protected) polyethylene plastic tube 3 m long by 2×2 m square and open at each end. The bottom end was supplied with a welded seam into which lengths of $3 \times 25 \times 25$ mm mild steel angle were inserted and tied together with polypropylene rope to make the bottom frame. The liners were rolled up and transported to the support frame by canoe. The liners were opened and then slowly lowered into the water until the bottom frame was in contact with the sediments. The top of the liner was stapled to the support frame, and sufficient slack was left in the sides to allow for movement resulting from changes in water level and/or wave action. SCUBA inspection of the enclosures showed that the bottom frames had penetrated into the sediments. In order to measure adsorption of pesticide to the walls of the enclosure, six polyethylene strips measuring $1000 \times$ 50 mm and having a total surface area of 1000 cm² were stapled to a wooden float and hung in the corral with the aid of small lead weights. The wood float was attached to the support frame with the aid of a short length of galvanized wire.

Treatment. Enclosures were treated with hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione] as Velpar L formulation (25% AI), with 2,4-D [(2,4-dichlorophenoxy)acetic acid] as the isooctyl ester formulation (60% acid equivalent [AE]), and with triclopyr [[(2,3,5-trichloro-6-pyridyl)oxy]acetic acid] as Garlon 4E butoxyethanol ester formulation (48% AE) as indicated in Table I.

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Figure 1. Construction details of the sediment sampler.

Herbicides were applied to the enclosures on June 26, 1984, at 10h00. Treatment was by means of a surface spray applied by a portable hand-pump sprayer. Herbicide was added to 1 L of lake water in the sprayer, and the total contents were applied through a jet nozzle. Low concentrations were applied first, and the sprayer was rinsed with acetone before a different chemical was used. In this way the amount applied was precisely known, contamination was reduced, and drift was minimal.

Sampling. Pretreatment samples were taken from enclosures 1, 13, and 14 and consisted of 3 L of water taken with an integrated water sampler (Solomon et al., 1982) from the surface to 2.2-m depth. A total of three integrated samples were taken at intervals across the enclosure and pooled for analysis. After surface application of the herbicides, a concentration gradient would have existed in the enclosures. Other work (Solomon et al., 1986) has shown that mixing in the enclosures is rapid, and it is likely that the chemicals were homogeneously distributed within 24 h of application. Sediment cores were taken with a sediment corer attached to a 3-m handle (Figure 1), placed in glass jars, and frozen until analysis. Starting on day 1 (24 h after treatment), water, sediment, and plastic strip samples were taken at predetermined intervals. Water, temperature, and dissolved oxygen concentrations were also determined and notes made of any unusual visual observations.

Analysis. Analytical methods were chosen to maximize recovery of parent herbicides. However, in the case of hexazinone, analyses of water and sediments revealed peaks corresponding to two metabolites: 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione and 3-cyclohexyl-6-(methylamino)-1methyl-1,3,5-triazine-2,4(1H,3H)-dione. Recovery efficiency for these metabolites was variable, and data are notpresented. Because of logistical problems, certain aspectsof analysis were conducted in the field. Upon completionof sampling, all sediment and strip samples were frozenat <math>-17 °C. The plastic strips were first wrapped in aluminum foil and placed in individual labeled plastic bags. Hexazinone water samples were transported to the field laboratory for immediate processing. One-liter aliquots were extracted with chloroform $(3 \times 25 \text{ mL})$. The combined chloroform washes were dried through anhydrous sodium sulfate and stored in 75-mL screw-top tubes until analysis could be performed.

The method of Scott et al. (1982) was used to extract triclopyr and 2,4-D from water. A 1-L aliquot was acidifed with 2-3 mL of 37 N H_2SO_4 . Under a vacuum of 25-35 cmHg, each water sample was then drawn through a 25-mL buret containing 8 g of a mixture of 50:50 XAD-2 and XAD-7 resins at a flow rate of approximately 40-50 mL/min. The resin was then flushed with three aliquots of diethyl ether (15, 15, 5 mL). This was partitioned three times into petroleum ether (15, 5, 5, mL); the ether fractions were dried through sodium sulfate and collected in 75-mL screw-cap test tubes. Samples were stored in this state until further analysis.

In the laboratory the chloroform extracts of hexazinone were transferred to a round-bottomed flask and evaporated to dryness on a rotary evaporator and water bath at 45 °C. The residue was dissolved in ethyl acetate, transferred to a test tube, and evaporated to a small volume in a stream of dry nitrogen. The sample was then brought to a known volume with ethyl acetate in a volumetric flask. Further concentration or dilution was carried out as appropriate for analysis.

The ether extracts of triclopyr and 2,4-D were evaporated to near-dryness in a stream of nitrogen then taken up in methanol. The samples were methylated with 2 mL of BF₃ reagent as catalyst while being heated in a water bath at 90 °C for 45 min. A liquid-liquid partition was performed with three aliquots of petroleum ether (5, 5, 5 mL). The ether fractions were dried with anhydrous sodium sulfate and collected in 20-mL screw-cap tubes containing 1 mL of isooctane. The ether was evaporated off in a stream of dry nitrogen, leaving the isooctane as a keeper. The sample was quantitatively transferred to a volumetric flask and made up to a known volume with isooctane. Samples were stored in this state until analysis.

Sediment samples from hexazinone enclosures were frozen in glass jars containing 200 mL of chloroform. At the time of extraction the sample was thawed and the chloroform and water phases were removed by filtration through Whatman glass fiber filter paper on a Buchner funnel. This liquid phase was quantitatively transferred to a separatory funnel and the chloroform separated into a round-bottomed flask and evaporated to dryness on a rotary evaporator and water bath at 45 °C. The water was then extracted with 2×200 mL of ethyl acetate, which was dried through a funnel packed with anhydrous sodium sulfate into the flask containing the residues from the chloroform extraction. This extract was evaporated close to dryness on a rotary evaporator and water bath at 45 °C, transferred to a volumetric flask, and made up to a known volume with ethyl acetate.

A 25-g aliquot of the sediment phase was placed in a centrifuge tube with 15 mL of distilled water and shaken for 15 min. Acetone (60 mL) was added and the tube shaken for a further 15 min and then centrifuged for 10 min at 3000g. The supernatant was decanted and filtered through Whatman No. 1 filter paper. The sediment was then resuspended in 75 mL of acetone-water (80:20), shaken for 2 min, centrifuged, and filtered. This was repeated once more, the combined extracts were quantitatively transferred to a round-bottomed flask, and the acetone was removed by evaporation on a rotary evaporator and water bath at 45 °C. The water was transferred to a separatory funnel and cleaned up by partitioning with

 3×50 mL of hexane which was discarded. The water fraction was extracted with 3×75 mL of chloroform, which was dried through a funnel packed with anhydrous sodium sulfate, collected in a round-bottomed flask, and evaporated to dryness on a rotary evaporator and water bath at 45 °C. The residue was dissolved in 50 mL of acetonitrile and further cleaned up by partitioning with 2×50 mL of hexane which was discarded. The acetonitrile was quantitatively transferred to a round-bottomed flask, evaporated to dryness on a rotary evaporator and water bath at 45 °C, redissolved in ethyl acetate, and made up to a known volume with ethyl acetate.

Sediment samples from the 2,4-D corrals were thawed, weighed, and homogenized for 1 min in a Waring blender. A 200-g aliquot of the sediment was extracted with 200 mL of acidified methanol by shaking for 1 h on a table shaker. After centrifugation at 750g for 7 min, the supernatant was filtered through Whatman glass fiber filter paper. The extract was reduced to a small volume on a rotary evaporator on a water bath at 45 °C and partitioned with 50, 25, and 25 mL of diethyl ether. The ether fraction was dried through anhydrous sodium sulfate and evaporated to near-dryness on a rotary evaporator on a water bath at 45 °C, and the residue was quantitatively transferred to a 15-mL test tube with methanol. The residue was methylated by 2 mL of BF₃ reagent as for the water samples above.

Sediments from the triclopyr-treated corrals were extracted as for 2,4-D above. The extract was reduced to a small volume on a rotary evaporator on a water bath at 45 °C, 50 mL of acidified 3% aqueous sodium chloride was added, and the herbicide residues were partitioned with 50, 25, and 25 mL of diethyl ether. The ether fraction was dried through anhydrous sodium sulfate and evaporated to near-dryness on a rotary evaporator on a water bath at 45 °C, and the residue was transferred to a 15-mL test tube with methanol. The volume was adjusted to 5 mL with methanol and 1 mL of 0.1 N sodium hydroxide in methanol added. The mixture was hydrolyzed on a water bath at 65 °C for 30 min after which it was allowed to cool and 1 mL of 0.1 N sulfuric acid added. The samples were methylated and further analyzed as for water above.

Plastic strips from the hexazinone corrals were placed in a 500-mL separatory funnel containing 25 mL of distilled water. The strip was extracted three times with 40, 20, and 20 mL of chloroform, respectively. These fractions were dried through anhydrous sodium sulfate and analyzed as for water.

In the case of triclopyr and 2,4-D, each strip was placed in a 500-mL separatory funnel containing 25 mL of distilled water. Strips were extracted with 3×25 mL of diethyl ether with vigorous shaking for 2 min. The ether fractions were dried through anhydrous sodium sulfate, evaporated to near-dryness, transferred to a 20-mL test tube with about 1 mL of methanol, methylated, and cleaned up as for water above.

All hexazinone samples were analyzed on a Varian 6000 gas chromatograph equipped with an N/P detector and autosampler. The following conditions were used: column, 600 × 2 mm; packing, 10% SP2250DA on 100/120 Supelcoport; injector, 260 °C; column, 240 °C from 0 to 2.5 min and then increased by 10 °C/min to 280 °C with a hold for 3 min at this temperature; detector, 300 °C; carrier gas, nitrogen (ultrahigh purity) at 33 mL/min; detector gas, hydrogen (prepurified) at 45 mL/min; air (zero gas grade), 175 mL/min. Samples were injected in a volume of 2 μ L and standards containing hexazinone and metabolites A and B were run between each analytical sample.

Table II. Mean Recovery Efficiencies

		_	triclopyr
matrix	hexazinone	2,4-D	acid
lake water			
concn, $\mu g/L$	10	100	100
recovery (±SE)	82.9 (0.95)	78.6 (4.17)	66.3 (1.96)
$LOD,^a \mu g/L$		0.005	0.005
$LOQ,^{a} \mu g/L$		0.01	0.01
sediments			
concn, µg/kg	80	100	100
recovery $(\pm SE)$	70.3 (0.18)	62.5 (1.7)	70.9 (1.25)
$LOD,^{a} \mu g/kg$		0.02	0.01
$LOQ,^{a} \mu g/kg$		0.04	0.02
strips			
concn, µg/strip	1	204	49
recovery $(\pm SE)$	b	47.8 (0.37)	73.0 (0.52)
$LOD^{a} \mu g/strip$		0.02	0.01
LOQ,ª µg/strip		0.05	0.02

^aLimit of detection and limit of quantitation. ^bConcentrations of coextractives were so high that determination of recovery was not possible.

Residue concentrations were quantified by comparison of peak height to mean peak height of standards run before and after each analytical sample. A Varian CDS 402 data system was used for quantification.

In the case of 2,4-D and triclopyr a Varian 3700 gas chromatograph fitted with an electron-capture detector was used under the following conditions: column, $2000 \times$ 3 mm; packing, 3% OV 17 on 80/100 Gas-Chrom Q; injector, 200 °C; column, 180 °C; detector, 300 °C; carrier gas, nitrogen (ultrahigh purity) at 30 mL/min. The output from the electron-capture detector was integrated with a Varian 402 data system, and results were calculated in reference to a standard curve and standards run on a daily basis.

Concentrations of herbicides in the enclosures were corrected for recovery efficiency and converted into total amount in each compartment using a nominal volume of $10\,000$ L of water, a sediment area of 4 m^2 , and a wall area of 20 m^2 .

RESULTS AND DISCUSSION

Recovery efficiencies of hexazinone, triclopyr, and 2,4-D from spiked, field-collected samples are shown in Table II.

Mean temperature and oxygen concentrations in the 2,4-D, the triclopyr enclosures, and the lake itself did not differ during the duration of the study. Compared to this, there was a dose-dependent reduction in oxygen concentration in the hexazinone corrals for about 2 weeks after treatment (Figure 2). This suggested that hexazinone caused a reduction in the productivity of the enclosure, most probably through inhibition of photosynthesis in algae since macrophytes were absent. Since oxygen concentration was only measured 0.5 m from the surface, depression of productivity may have been larger than indicated because of the influence of atmospheric oxygen. The differences in oxygen concentration decreased with time after treatment and levels in the treated enclosures appeared to recover by 21 days posttreatment.

Hexazinone concentrations in water (Figure 3) declined rapidly at both application rates. At the lower application rate hexazinone was undetectable 21 days and at the higher rate 42 days after application. Metabolites were detected between days 2 and 14 at the low and between day 2 and 42 at the high rate of application. From this it appeared that hexazinone dissipated by means of chemical, photolytic, and/or biologically mediated breakdown. Residues of hexazinone in the sediments were low (Figure 3). Hexazinone residues could not be analyzed for in the strips



Figure 2. Temperature and oxygen concentration in the hexazinone-treated enclosures. Each point represents the mean of three enclosures; standard errors are indicated by the vertical bars.



Figure 3. Dissipation of hexazinone from enclosures. Each point represents the mean of three enclosures; standard errors are indicated by the vertical bars.

of side wall material, but its relatively high water solubility as well as the observation that this herbicide was not absorbed in plastic sampling bottles (Feng, J., Canadian Forest Pest Management Institute, personal communication) suggests that the material is unlikely to adsorb to sediments, plastic, or organic material. The hypothesis of lack of major adsorption to sediments was confirmed in this study. Total dissipation of hexazinone from the corrals was very similar to that in the water (Figure 3).

The method of analysis of 2,4-D and triclopyr did not allow for differentiation of ester and free acid, and all results are reported as total acid equivalent. The dissipation of 2,4-D (Figure 4) was relatively rapid and followed similar trends at both concentrations. Levels found in water on day 1 were somewhat lower than on day 2. This is thought to be due to a lack of adequate mixing in the short time period between treatment and sampling. Within 15 days, less than 5% of the applied herbicide remained in the water. Of interest was the relatively large proportion of the pesticide adsorbing to the walls of the enclosures (Figure 4). In similar studies with methoxychlor (Solomon et al., 1986) adsorption to the sides of enclosures



Figure 4. Dissipation of 2,4-D from enclosures. Each point represents the mean of three enclosures; standard errors are indicated by the vertical bars.

was also noted. This phenomena was not noted with water-soluble pesticides such as atrazine (Herman et al., 1986) and, in this case, is most likely due to adsorption of the relatively water-insoluble isooctyl ester. Slow release of the ester and its hydrolysis in water to give the free acid probably accounts for the apparently long but low-level persistence of the herbicide in the water. Adsorption of 2,4-D to sediments was low in comparison to adsorption to the sides (Figure 4). While this is unexpected in relation to adsorption to the walls, the relatively larger area of the enclosure wall must be taken into consideration. A similar relationship has also been observed with methoxychlor (Solomon et al., 1986).

Dissipation of triclopyr from water (Figure 5) was rapid, and the herbicide could not be detected in water from day 42 onward. Rates of dissipation from water were similar at both levels of application, and levels were below 5% of applied within 15 days. The amount of pesticide absorbed to the sides of the enclosures was low (Figure 5) and appeared to dissipate more rapidly than was the case with 2.4-D. The ester moiety in this case was butoxyethanol, which, being of shorter chain length, would be expected to be more polar and more rapidly subjected to hydrolysis than the isooctyl ester of 2,4-D. This may account for the lower absorption to and also more rapid dissipation from the plastic. Sediment analyses (Figure 5) showed that a relatively small, but variable, proportion of applied pesticide was adsorbed. Dissipation from the sediments was, as expected, relatively slower than from water but does not indicate persistence that would result in significant carry over for long periods of time. With respect to triclopyr, these observations confirm the predicted short half-life of both triclopyr and its butoxyethanol ester (McCall and Gavit, 1986). Using laboratory-derived photolysis data, they predicted midsummer half-lives in the range of 1-3 days at latitude 50° in clear water. The rapid photolysis of the ester in the water column may also account for the lower than expected adsorption to sediments.

Linear regression analysis of log concentration vs time gave estimates of half-life (DT_{50}) and time to 95% dissi-



Figure 5. Dissipation of triclopyr from enclosures. Each point represents the mean of three enclosures; standard errors are indicated by the vertical bars.

Table III. Estimated DT_{50} and DT_{95}^{a} Times for Hexazinone, 2,4-D, and Triclopyr in Lake Water

dose, kg/ha	equation ^b	r^2	DT ₅₀ , days	DT ₉₅ , days				
Hexazinone								
4.0	$T = (\log x)(-9.57) + 20.50$	0.90	3.8	13.4				
0.4	$T = (\log x)(-7.67) + 16.75$	0.85	3.7	11.4				
2.4 - D								
2.5	$T = (\log x)(-15.66) + 34.31$	0.73	7.8	23.5				
1.0	$T = (\log x)(-19.63) + 37.92$	0.77	4.5	24.2				
Triclopyr								
3.0	$T = (\log x)(-11.60) + 23.97$	0.97	4.3	15.9				
0.3	$T = (\log x)(-11.21) + 22.81$	0.57	3.8	15.0				

^aTimes for 50% and 95% dissipations, respectively. ^bFrom a least-squares regression of log concentration (x) vs time (T).

pation (DT_{95}) based on the assumption of pseudo-first-order kinetics (Table III).

Adsorption of water-insoluble pesticides to the sides of the enclosures is an artifact but is unavoidable. It could possibly be reduced if other, less absorptive (Sharom and Solomon, 1980) although more expensive, materials such as Teflon were used as wall material. Adsorption and desorption of pesticides onto the walls and sediments of the enclosures probably reaches equilibrium quite rapidly. As such and provided it is measured, adsorption to the walls could be factored out in the application of data from enclosures to environmental models.

It appears that, under actual conditions existing in a typical Ontario forestry environment, hexazinone and triclopyr are relatively nonpersistent in water and are not bound to sediments to any great extent. There is also little practical difference between hexazinone, the butoxyethanol ester of triclopyr, and the isooctyl ester of 2,4-D with respect to either persistence or distribution in the aquatic system although hexazinone appears to dissipate somewhat more rapidly than 2,4-D.

While not specifically assessed in this study, it is probable that photolysis was a major factor in the dissipation of all three herbicides from the enclosures. From the point of view of environmental impact, the relatively short persistence of these chemicals suggests that their effects in the aquatic ecosystem, if any, are likely to be short-term and acute in nature. The depression of photosynthesis observed with hexazinone was lower and of shorter duration than has been reported for atrazine (Larsen et al., 1986; Hamilton et al., 1987) and would not be expected to have either major or long-term secondary effects in the biota. The short duration of this effect is undoubtedly due to the rapid dissipation of this herbicide; it should, however, be noted that macrophytes were not present in this study and that significant effects on these aquatic plants have been reported in the case of atrazine (Larsen et al., 1986).

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