Persistence and Leachability of Glufosinate-Ammonium in a Northern Ontario Terrestrial Environment

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The degradation and leaching potential of the herbicide [^{14}C]glufosinate-ammonium [the ammonium salt of DL-homoalanin-4-yl(methyl)phosphinic acid] in a northern Ontario forest environment were examined. The DT_{50} and maximum leaching depth were 4.3 days and 10 cm (humic layer), respectively. [^{14}C]MPPA-3 (3-methylphosphinylpropionic acid) and [^{14}C]MPAA-2 (2-methylphosphinylacetic acid), the two main metabolites of glufosinate-ammonium, also did not leach beyond 10 cm. At 32 days postapplication, approximately 10−20% of the parent and metabolites remained in the soil, but by the following season (day 295) residue levels had declined to near zero.

Keywords: *Glufosinate-ammonium; forestry; persistence; leaching potential*

INTRODUCTION

Glufosinate-ammonium [the ammonium salt of DL-homoalanin-4-yl(methyl)phosphinic acid] (Figure 1) is currently being evaluated as a site preparation herbicide. It is a postemergence, nonselective herbicide with a maximum label rate of 1.7 kg of active ingredient (ai)/ha. Glufosinate-ammonium is a synthetic herbicide, related to the natural product bialaphos produced by *Streptomyces viridochromogenes* L. (Duke and Lydon, 1987), both compounds containing phosphinothricin as the active ingredient. Phosphinothricin is a potent inhibitor of the enzyme glutamine synthetase (Manderschied and Wild, 1986; Wild and Manderschied, 1984), which controls ammonia assimilation in higher plants. Following phosphinothricin-induced inhibition of the enzyme, toxic levels of ammonia accumulate and lead to plant death (Ray, 1989; Wild and Manderschied, 1984; Lea et al., 1984). Various dissipation pathways exist for pesticides in a soil environment, with the predominant ones being microbial degradation, volatilization, photodegradation, and chemical hydrolysis (McEwen and Stephenson, 1979). For glufosinate-ammonium, microbial degradation is the most important (Bartsch and Tebbe, 1989; Tebbe and Reber, 1988), with the degradation rate being dependent on soil characteristics and environmental conditions. As conditions become more conducive to microbial growth, the degradation of organic pesticides will increase. Optimum conditions would be a highly organic, moist soil, which would promote microbial growth and thus increase degradation rates (Behrendt et al., 1990). Glufosinate-ammonium degrades rapidly in a nonsterile environment such as soil with DT_{50} values being reported at 1−10 days in sandy loam soils (Gallina and Stephenson, 1992; Behrendt et al., 1990; Smith, 1989) and 15−25 days in clay and clay loam soils (Smith, 1989; Smith and Belyk, 1989). Field studies have indicated that glufosinate-ammonium rarely leaches below 10−15 cm in soil (Kubiak, 1992; Gallina, 1990; Smith and Belyk, 1989). Studies have shown the formation of two main metabolites during the degradation process (Figure 1). Specifically, the first main metabolite is 3-methylphosphinylpropionic acid (MPPA-3), which is degraded to 2-methylphosphinylacetic acid (MPAA-2) (Behrendt et al., 1990). These studies, however, were all carried out on mineral soils with only moderate organic matter content (1−6%).

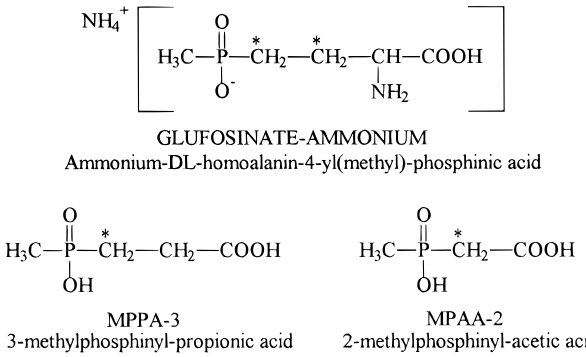

Figure 1. Glufosinate-ammonium and its two main metabolites. (A dot indicates ^{14}C in radiolabeled standards.)

From an environmental safety point of view, the short DT_{50} and minimal soil mobility make glufosinate-ammonium an appealing herbicide for potential use in forestry. However, the persistence and mobility of this compound must be tested specifically in a forestry situation before its use. The study presented here was conducted to examine the degradation and leaching potential of glufosinate-ammonium and its main metabolites in a northern forest ecosystem with more highly organic soils.

MATERIALS AND METHODS

Chemicals. [^{14}C]Glufosinate-ammonium, labeled in the two $-CH_2-$ carbon atoms, with a specific activity of 4.00 μCi/mmol was custom synthesized by New England Nuclear Research Products, Boston, MA. [^{14}C]MPPA-3 (specific activity of 1.01 μCi/mmol) and [^{14}C]MPAA-2 (specific activity of 0.96 μCi/mmol) labeled in the third and second carbon positions, respectively (Figure 1), analytically pure glufosinate-ammonium, MPPA-3, and MPAA-2 as well as formulated glufosinate-ammonium (Ignite,137 g of ai/L) were provided by AgrEvo Canada Inc., Regina, SK.

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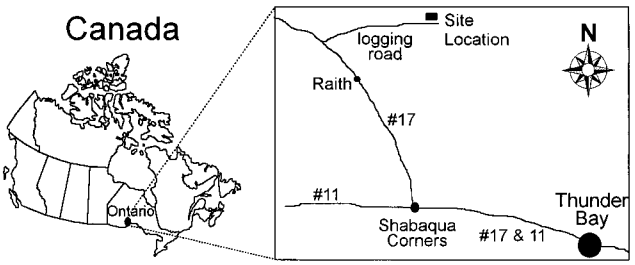

Figure 2. Experimental site location.

Table 1. Composition and Physical Characteristics of the Forest Soil Used in This Study

depth (cm)	soil type	% sand	% silt	% clay	% OM	pH	CEC[a]
0−10	humus	34.3	59.8	6.0	17.2	4.8	20.8
10−30	sandy loam	35.5	61.5	3.0	3.8	5.2	7.9

[a] Milliequivalents per 100 g of oven-dry soil.

Experimental Site. The site was located approximately 100 km west of Thunder Bay, ON (89° 56′ W, 48° 52′ N) (Figure 2) on a forest site 6 years after clear cutting. The physical characteristics of the soil located at the experimental site are summarized in Table 1.

Site Preparation. Replicate plot areas ($n = 4$) were staked out 10 m apart, and all grass awas cut and removed. Forty (10 sampling times × 4 replicates) stainless steel tubes (10 cm o.d. × 30 cm length) were driven into the ground, leaving the top 1−2 cm exposed. Debris around each tube was removed to prevent water from running into the top of the tube. These tubes were left to equilibrate for approximately 2 weeks prior to herbicide application. Two days prior to the application date, four tubes (one per replicate) were carefully removed, a leachate trap was attached, and then the tube was returned to its original location. These tubes were assigned the last sampling time.

Herbicide Application. On the application day (August 22, 1993), 0.917 mL of Ignite was added to a total volume of 80 mL of distilled water, and to this solution was added 200 μCi of [^{14}C]glufosinate-ammonium. After homogenization, 2 mL (equivalent to a 5 μCi and 4 kg of ai/ha application of radiolabeled and formulated glufosinate-ammonium to the surface of each tube) was added in a dropwise fashion to the surface of each tube in cross-hatched pattern to ensure even coverage. A 1 cm perimeter within each soil column was left untreated to avoid the contamination of deeper soil increments via the tube−soil interface. Each tube was then covered and the entire site over-sprayed with Ignite at a rate of 4 kg of ai/ha to simulate conditions that would be encountered in an actual forest application.

Confirmation of the nominal radioactive rate (5 μCi) on day 0 was conducted via oxygen combustion using a Model OX300 biological oxidizer (R. J. Harvey Instrument Corp., Hillsdale, NJ). Analysis revealed actual levels of 3.35, 3.17, 2.26, and 2.47 μCi for replicates 1−4, respectively. The difference between the first two replicates and the last two can be explained by human error. The chemical was applied by two researchers, one for the first two replicates and one for the last two. Therefore, some of the difference is likely due to variations in applicator technique. The remainder of the error could be attributed to unequal preparation of the two stock solutions. All four replicates were below the target rate of 5 μCi, which was likely due to the highly viscous nature (adhered to the wall of the 2 mL pipet used in the application) of the application mixture. Due to these rate differences, each soil core harvested was compared to its respective replicate number for the determination of the percentage of applied radioactivity remaining.

Sampling. The sampling intervals used in this study were 0, 2, 4, 6, 8, 10, 12, 18, 32, and 295 days postapplication. On each respective sampling day, a soil core from each of the four replicates was carefully removed from the ground and sand was added to the top of each tube to fill any air voids that might exist. This was done to prevent mixing of the top soil layers during transport. The tubes were sealed with black plastic and tape and placed immediately on ice until a −20 °C storage facility could be reached (approximately 8 h), where they were stored until analysis. On the final sampling day (the following season) the leachate traps were removed and the leachate was assayed for radioactivity by scintillation.

Extraction from Soil. Figure 3 shows a detailed description of the method used to extract and quantify glufosinate-ammonium and its main metabolites from highly organic soils. Since the final sample volume varied slightly after the extraction procedure, total radioactivity in each sample was determined by liquid scintillation. Extraction and analysis methods were validated by fortifying blank soils taken from the spray site prior to chemical application. Three validation groups were set up to mimic the estimated degradation pattern of glufosinate-ammonium over time. The first group (initial) represented herbicide levels before any degradation occurred. The second (middle) and third groups (end) represented the estimated amounts and proportions of both the parent compound and its metabolites midway and near the completion of the degradation process. Fortification was completed using mixtures of radiolabeled and analytical grade glufosinate-ammonium to yield relevant application rates and total radioactivity as shown in Table 2.

HPLC Analysis. Samples were analyzed using a Shimadzu SCL-6B HPLC (Shimadzu Corp., Kyoto, Japan) equipped with a beta radioactive flow detector (Canberra Packard, Tampa, FL). The mobile phase was a 50 mM KH_2PO_4 buffer adjusted to pH 2.1 using H_3PO_4 with 10% methanol (Optima grade, Fisher Scientific, Whitby, ON) as eluant. Samples were passed through a silica based guard column (5 mm × 45 mm) with 30−40 μm pore size (Scientific Products and Equipment, Concord, ON) followed by a Spherisorb SAX column (10 mm × 250 mm) with 5 μm pore size (Phenomenex, Torrance, CA) at a flow rate of 2 mL/min and a fluor to buffer ratio of 4:1. A

Table 2. Method Validation for the Extraction of [^{14}C]Glufosinate-Ammonium from a Forest Soil at Various Time Intervals after Application

	initial hot (μCi)/cold (kg/ha) applied[b]			radioactivity recovered (μCi)[c]				
soil spiking group[a]	glufosinate-ammonium	MPPA-3	MPAA-2	glufosinate-ammonium	MPPA-3	MPAA-2	extraction efficiency[d]	CV
initial	5.0/4.0	0/0	0/0	3.8 (0.026)	0.13 (0.027)	0 (0)	79 (3.8)	4.9
middle	2.5/2.0	0.5/0.4	0.1/0.08	2.1 (0.0075)	0.51 (0.018)	0.062 (0.012)	87 (1.5)	1.8
end	0.5/0.4	0.1/0.08	0.5/0.4	0.40 (0.0017)	0.081 (0.0034)	0.46 (0.015)	87 (3.6)	4.1
control	0/0	0/0	0/0	0 (0)		0 (0)		

[a] Group refers to time in relation to the field sampling period (i.e., initial is prior to any degradation; middle and end are mid-way and near completion of the predicted degradation process, respectively). [b] Amounts were designed to represent the predicted dissipation of glufosinate-ammonium and its transformation into metabolites over the sampling period. Hot refers to radiolabeled compound, and cold refers to analytical grade compound (i.e., for glufosinate-ammonium in the initial group, 5.0 μCi of radiolabeled compound and 4.0 kg/ha of analytical grade compound were applied). [c] Numbers in parentheses represent standard error. [d] Extraction efficiency expressed as a percentage, and numbers in parentheses represent standard error.

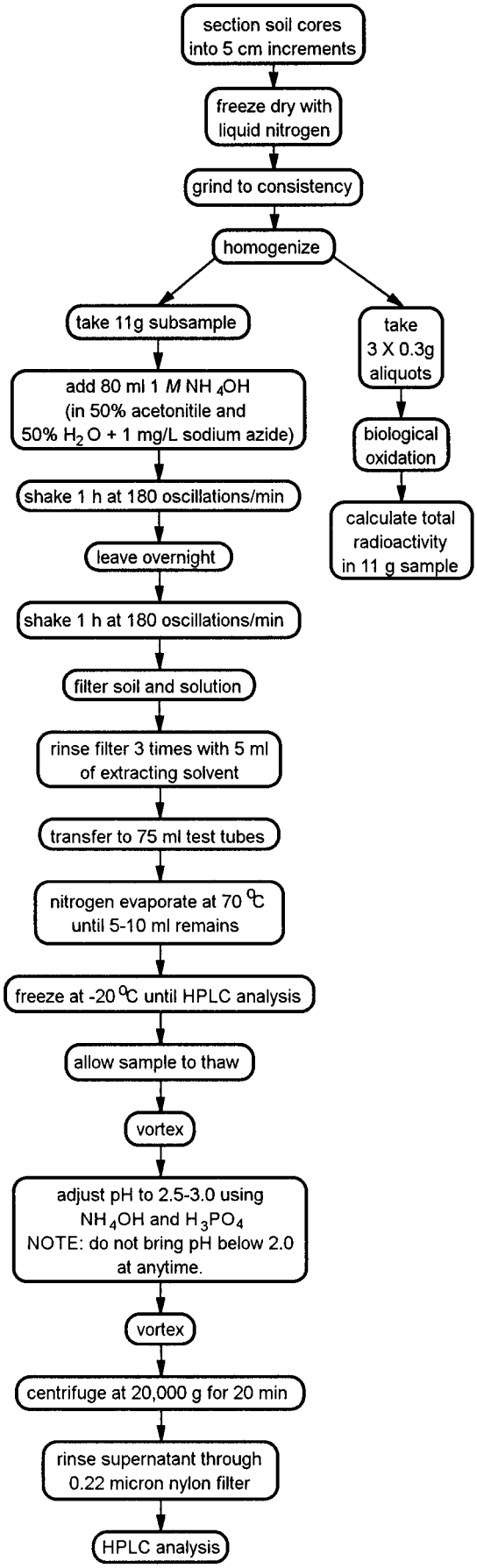

Figure 3. Flow chart of methodology for the extraction of [^{14}C]-glufosinate-ammonium from highly organic soils.

30 min isocratic run was used giving retention times of 9.83, 13.83, and 20.00 min for glufosinate-ammonium, MPAA-3, and MPAA-2, respectively (Figure 4).

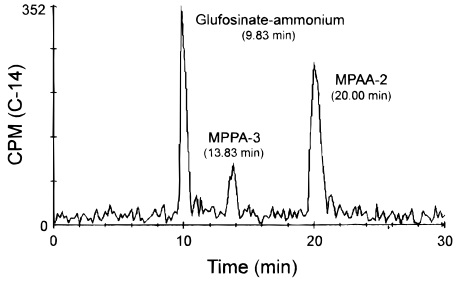

Figure 4. HPLC chromatogram showing retention times of [^{14}C]glufosinate-ammonium and its two main metabolites.

Storage Stability of Samples. Soil cores were stored for 12 months prior to analysis; therefore, a storage stability study was conducted. Twenty (4 replicates × 5 sampling times) plastic containers (4.5 cm o.d.) were filled with 25.0 g (11.4 g dry weight) of soil taken directly from the experimental site. Each container was spiked with 0.31 μCi of [^{14}C]glufosinate-ammonium and 4.2 μL of formulated glufosinate-ammonium (equivalent to a field application rate to a soil core of 2.5 μCi and 4.0 kg of ai/ha, respectively). Each container was sealed and stored at −20 °C until analysis. Samples were removed from storage at 0, 5, 7, 10, and 12 months postapplication, and the percentage of parent compound remaining was determined via HPLC.

Statistical Analysis. For the storage stability study, linear regression ($p < 0.05$) analysis was used to determine if glufosinate-ammonium degraded during storage. Nonlinear regression was used to fit the field dissipation data to the double-exponential decline model

$$y = ae^{(-bx)} + ce^{(-dx)}$$

where a is the intercept of the first exponential, b is the slope of the first exponential (indicates initial rate of dissipation), c is the intercept of the second exponential, d is the slope of the second exponential (indicates subsequent rate of dissipation), y is the percent of applied radioactivity, x is days postapplication, and e = euclid = 2.7183. The model was fitted to the data using SigmaStat (Jandel Corp., 1995) and was considered acceptable if the b parameter was significant ($p < 0.05$), if the ratio of regression sums of squares (RSS) to uncorrected total sums of squares (TSS) was high (>0.75), and if residuals generally complied with standard regression assumptions (Draper and Smith, 1981). This model has the ability to show the rapid decline and subsequent decreased degradation rate often observed during microbial degradation. This equation was then used to calculate the DT_{50} of glufosinate-ammonium ($DT_{50} = x$ when $y = a/2$).

RESULTS

Extraction Method Validation. The radioactivity recovered for each soil spiking group is shown in Table 2. Extraction efficiencies were 79 ± 3.8 (initial group), 87 ± 1.5 (middle group), and 87 ± 3.6% (end group) with coefficients of variation (CV) of 4.9, 1.8, and 4.1, respectively. The initial group was statistically ($p < 0.05$) different from the middle and end groups. It is, however, unlikely that this will affect the field data results since a subsample from each soil section was biologically oxidized prior to extraction to determine total radioactivity present and then extraction and analysis on HPLC was conducted to get the relative percentages of parent and main metabolites. These percentages were then used to back-calculate actual amounts.

Storage Stability. Linear regression ($y = 96.9 + 0.00139x$; $p = 0.99$; $F < 0.001$; $r^2 < 0.1$) analysis revealed that no significant degradation of glufosinate-ammonium to its main metabolites occurred during 12 months of storage (Figure 5). The poor r^2 value is attributable to the dip in recovery at 6 months of storage time, which

Table 3. Radioactivity Recovered as Parent and Main Metabolites from the Top and Bottom Portions of the Humus Layer of a Northern Ontario Forest Soil Treated with [^{14}C]Glufosinate-Ammonium

			% of applied radioactivity[a]					
			glufosinate-ammonium		MPPA-3		MPAA-2	
date	days since appln	rainfall since last sampling (mm)	0−5 cm[b]	5−10 cm	0−5 cm	5−10 cm	0−5 cm	5−10 cm
Aug 22, 1993	0	0	87 (4.9)	–[c]	13 (4.1)	–	–	–
Aug 24, 1993	2	14	49 (5.4)	6 (2.4)	24 (3.7)	4 (1.9)	3 (1.3)	–
Aug 26, 1993	4	21	37 (7.8)	3 (0.8)	24 (1.9)	5 (1.3)	5 (1.4)	2 (1.3)
Aug 28, 1993	6	4	16 (3.8)	4 (1.7)	18 (3.3)	12 (2.8)	8 (2.2)	8 (1.0)
Aug 30, 1993	8	9	24 (5.0)	3 (1.4)	22 (3.1)	8 (3.2)	12 (3.8)	4 (1.6)
Sept 1, 1993	10	0	12 (2.2)	5 (1.3)	14 (3.4)	10 (1.0)	8 (0.7)	11 (2.3)
Sept 3, 1993	12	8	13 (1.6)	8 (3.7)	12 (1.4)	11 (1.1)	7 (1.8)	11 (2.5)
Sept 9, 1993	18	29	12 (0.6)	2 (0.5)	9 (1.6)	7 (0.3)	10 (3.6)	12 (3.4)
Sept 23, 1993	32	nd[d]	13 (2.8)	1 (0.8)	12 (2.4)	4 (1.2)	12 (1.1)	7 (2.5)
June 12, 1994	295	nd	–	–	–	–	–	–

[a] Average of four replicates. Numbers in parentheses represent standard error. [b] Depths from 10 to 30 cm not shown since they contained <5% of applied radioactivity as determined by oxygen combustion of soil samples. [c] Percent recovered of parent and metabolites <5% of applied radioactivity. [d] Not determined.

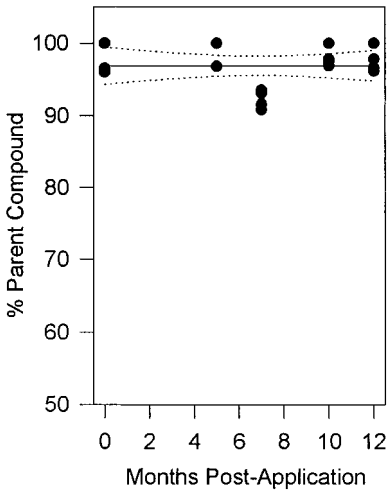

Figure 5. Linear regression results on the effect of 12 months of storage (−20 °C) on the stability of [^{14}C]glufosinate-ammonium. There was no significant ($p = 0.99$) degradation of parent compound over this period.

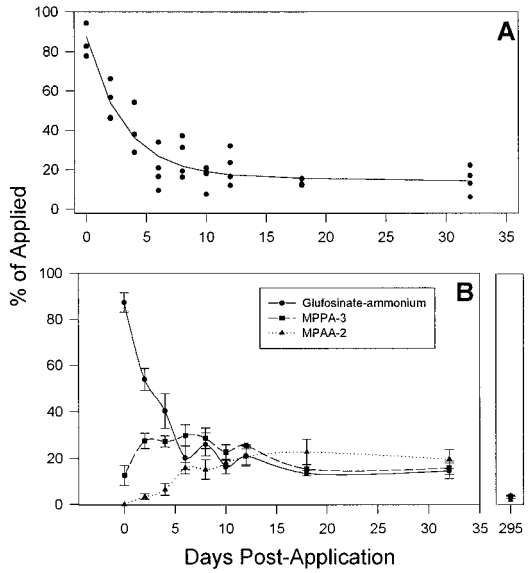

Figure 6. Nonlinear regression ($y = 70.4e^{(-0.322x)} + 17.1e^{(-0.00554x)}$; $F = 79.8$; $p < 0.001$) results (A) for the dissipation of [^{14}C]glufosinate-ammonium and its relationship to the degradation to its two main metabolites (B) over the 32-day observation period and the following season (day 295).

is likely an artifact of how efficiently samples were extracted at this time.

Dissipation and Leaching Potential. Figure 6A shows the nonlinear regression of the degradation of [^{14}C]glufosinate-ammonium over time fitted to a double-exponential decline model ($y = 70.4e^{(-0.322x)} + 17.1e^{(-0.00554x)}$; $F = 79.8$; $p < 0.001$). The DT_{50} of glufosinate-ammonium as determined from this model was 4.3 days. Figure 6B shows the relationship between the degradation of [^{14}C]glufosinate-ammonium, [^{14}C]-MPPA-3, and [^{14}C]MPAA-2 over the observation period. Data obtained for each soil increment were pooled together in this figure to show depth-independent degradation. Some degradation (13 ± 4.1%) of the parent compound to the first main metabolite ([^{14}C]-MPPA-3) was observed in the day 0 samples. By 2 days postapplication, an increase in the amount of [^{14}C]-MPPA-3 was observed, which coincided with a decrease in [^{14}C]glufosinate-ammonium. By 4 days postapplication, an increase in [^{14}C]MPAA-2 was observed. The relative timing of formation and subsequent degradation of [^{14}C]MPPA-3 and subsequent formation of [^{14}C]-MPAA-2 are consistent with the proposed degradation pathway (Gildemeister, 1988). Residue levels for parent and metabolites remained at 10−20% of applied radioactivity until 32 days postapplication. By the following season (day 295) residues levels were near zero.

Table 3 shows the radioactivity recovered as parent and main metabolites from the humus layer of each soil core. Consistent with previous studies, there was slight evidence of relatively greater mobility of metabolites as compared to the parent compound. However, the high organic matter (OM) and cation exchange capacity (CEC) of the humus layers in this soil prevented any substantial movement of either the parent (>95% in the 0−10 cm layer) or metabolites (>88% in the 0−10 cm layer). Below 10 cm in depth, not more than 5% of applied radioactivity was recovered. The majority (>95%) of this radioactivity was in the 10−15 cm increment and ranged from 1 to 3% of applied radioactivity. Less than 1% of the radioactivity was detected in the 15−20 cm increment, and no radioactivity was detected below this. Also, no radioactivity was detected in the leachate traps from the soil cores removed in the following season.

DISCUSSION

The degradation of [^{14}C]glufosinate-ammonium to [^{14}C]MPPA-3 observed in the day 0 soil cores may be related to the time it took to get the soil cores to a freezer or may also indicate some degradation prior to application. Time between sampling and frozen storage was maximally 8 h, and given the small DT_{50} (4.3 days) of glufosinate-ammonium, it is likely that some degra-

dation occurred during this time. Also, some of this observed degradation may have been as a result of hydrolytic degradation while the samples stood at ambient temperature overnight in basic solution.

Persistence and mobility are important criteria determining a herbicide's safety for environmental use. Glufosinate-ammonium has a small DT_{50} (4.3 days) in northern Ontario forest soils and does not leach beyond 10 cm in depth (<5% of applied radioactivity was detected below this). Another important consideration is whether the main metabolites of a pesticide are persistent or mobile. Neither MPPA-3 nor MPAA-2 leached beyond 10 cm in depth (<5% of applied radioactivity was detected below this), nor were residue levels carried into the next season. Although glufosinate-ammonium and its metabolites are potentially mobile (Gallina and Stephenson, 1992), they are degraded too quickly for significant mobility to be a concern. These results are consistent with data obtained by other researchers in different soil and environmental conditions (Gallina and Stephenson, 1992; Behrendt et al., 1990; Smith, 1988, 1989; Smith and Belyk, 1989).

From a soil persistence and mobility point of view, glufosinate-ammonium can be considered safe to use in forestry situations. There does not appear to be any potential for this herbicide to leach to ground water, and due to its small DT_{50}, exposures for soil biota would be limited to acute time frames and only for those organisms inhabiting or foraging in the upper 0−10 cm layers. The LC_{50} for earthworms treated with Basta (formulation of glufosinate-ammonium) is ≥1000 mg/kg (Dorn et al., 1992); therefore, it is unlikely that the indigenous earthworm population would be impacted. However, the safety of a herbicide to forestry environments depends on more factors than these. For example, impacts to soil microorganisms should also be considered as well persistence and impact in aquatic environments. Since aerial application of forestry herbicides is the usual method of delivery to the target site, these other factors must also be considered in the overall safety of glufosinate-ammonium to forest ecosystems.

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