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# Earthworms and the dissipation and distribution of atrazine in the soil profile

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## Abstract

The influence of earthworms *(Lumbricus terrestris* L.) on the persistence and transport of '$^{4}$C-labelled atrazine [2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine] in soil was studied in laboratory incubations using flask microcosms and packed columns. In soil microcosm incubations (12 or 30°C), [U-ring-$^{14}$C]atrazine was dissipated and mineralized more rapidly in soil that had been conditioned (preincubated) with earthworms (e.g. soil containing worm castings) than in soil without earthworms. Earthworms added to soil following herbicide application accelerated the formation of non-extractable (soil-bound) atrazine residues and reduced atrazine mineralization rates over 68 d, compared to soil that did not contain earthworms. In packed soil columns (24 cm x 6.3 cm i.d.), earthworms promoted the formation of non-extractable residues and modified the vertical distribution of herbicide residues. Following a 68-d incubation of soil columns (12°C) receiving a surface application of [U-ring-$^{14}$C]atrazine-sprayed corn leaves, total non-extractable radioactivity in soil columns containing earthworms was 21% greater than that in soils without earthworms. Earthworm consumption of the [U-ring-$^{14}$C]atrazine-sprayed corn leaves and subsequent activity translocated 60% of the total radioactivity below 4 cm. In contrast, more than 65% of the initially applied [U-ring-$^{14}$C]atrazine remained in the top 4 cm surface layer in columns containing no earthworms. Earthworms also influenced the distribution of herbicide residues in the soil matrix, depositing about twice as much MeOH-extractable radioactivity in their burrow linings as in the surrounding soil.


*Keywords:* Earthworms; Atrazine mineralization; Atrazine transport

## 1. Introduction

Atrazine[2-chloro-4-ethylamino-6-isopropyl-amino1,3,5-triazine] is one of the most heavily used herbicides in North America and is a frequently detected groundwater pollutant (Goodrich *et al.*, 1991; Liu *et al.*, 1996). Atrazine movement through the soil profile is influenced by a variety of factors including soil texture and structure. Particularly important is the abundance and distribution of macropores such as earthworm burrows and the voids left behind by decaying crop roots (Sigua *et al.*, 1995). Earthworm channels can serve as preferential flow paths which rapidly conduct agrochemicals during rainstorms (Sadeghi and Isensee, 1994). Most information on herbicide movement via earthworm burrows in soils is based on laboratory experiments where rainfall was applied to soil columns shortly after herbicide applications. Little attention has been paid to herbicides losses via burrow flow when rainfall is delayed.

Earthworms may move herbicides from the soil surface into the soil by burying crop residues during inter-storm periods. Earthworms feeding on herbicide-sprayed crop residues may deplete the herbicides remaining near the surface soil, thereby decreasing their availability for preferential transport through earthworm burrows.

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The earthworm species *Lumbricus terrestris* L. feeds predominantly on organic materials at the soil surface. After ingestion, these materials pass through the body and are deposited deeper in the profile as castings (Lee, 1985). Numerous investigations have shown that burrow linings contain a greater percentage of organic C, clay content, available nutrients and heavy metals compared to the surrounding soil (Tomlin *et al.*, 1992; Stehouwer *et al.*, 1993; Basker *et al.*, 1994). Likewise; the effects of *L. terrestris* on the chemical, physical and biological properties of soils have been shown to influence pesticide persistence, bioavailability and transport. Mallawatantri *et al.* (1996) demonstrated that mineralization rates of 2,4-D and carbofuran are greater in burrow linings than in soil matrix. Atrazine mineralization rates were greater in soils with *L. terrestris* than in soils without earthworms (Meharg, 1996). *L. terrestris* can enhance atrazine degradation in their digestive system (Chio and Sanborg, 1978) while pesticide transformation by earthworms has also been observed for several insecticidal compounds (Gilman and Vardanis, 1974; Stenersen *et al.*, 1974).

Earthworms, particularly *L. terrestris,* are more abundant in no-till agricultural soils than in corresponding tilled soils, because under no-till management there is little soil disturbance of earthworm habitat. Therefore, the effects of earthworms on pesticide fate and transport is likely to be more significant in no-till than in tilled agricultural soils. In no-till soils with large amounts of crop residues at the surface, Isensee and Sadeghi (1994) found that more than two-thirds of applied herbicides were intercepted by the crop residues and living vegetation. It follows that *L. terrestris* is likely to move significant portions of these herbicides into the soil by burying crop residues, or by ingesting and then egesting these materials at depth as castings. To date, there are no reports that relate the feeding activity of *L. terrestris* to the rate of herbicide transport from the soil surface into burrow linings. Our main objective was to estimate the effects of earthworms on the persistence and movement of atrazine in soil.

## 2. Material and methods

### *2.1. Soil, earthworms and crop residues*

A loamy Ap horizon (0 to 15 cm) of a Gobles soil at a field moisture content of 13% was collected in 1994 from a no-till corn field near Belmont, Ontario. The field was converted to no-till in 1989 and was continuously cropped to corn; previously it had been in a corn- corn-soybean rotation with fall mouldboard ploughing. Following an extraction procedure as reported in Topp *et al.* (1994), no atrazine residues were detected in the sampled soil (HPLC detection limit 25 ng $g^{-1}$ soil). Key physical and chemical soil properties of the soil include: 29% sand, 46% silt, 24% clay, 1.63% organic carbon, pH 6.35, CEC 12.38 cmol $kg^{-1}$ and an exchangeable K, Ca, Mg and Na of 0.47, 5.62, 1.56 and 0.07 cmol $kg^{-1}$ respectively. Soil texture was determined by the hydrometer method (Gee and Bauder, 1986), organic C by the modified Walkley-Black volumetric oxidation method (Allison, 1965), pH in a 1:1 soil to 10 mM $CaCl_2$ solution, CEC by the $NH_4OAC$ method (Chapman, 1965) and exchangeable K, Ca, Mg and Na according to Jackson (1958).

Mature *L. terrestris* were purchased locally and stored at 12°C. Worm digestive tracts were cleared of previously ingested contents by incubating them in soil containing corn leaf residues for 5 d before they were used in experiments.

Corn leaves were obtained from a pesticide-free field plot at Southern Crop Protection and Food Research Centre, London, Ontario. Leaves were pulverized into small pieces (2.5-7.5 $mm^2$) using a blender to produce a material more palatable for earthworms.

### *2.2. Chemicals*

A commercially available atrazine-metolachlor liquid formulation, Primextra® (label content: 153 g atrazine $L^{-1}$, 364 g metolachlor $L^{-1}$ and 10 g unidentified other active triazines $L^{-1}$; Ciba-Geigy Co., Greensboro, NC) was used. Primextra® stock solutions for experiments were prepared by diluting Primextra® with reverse osmosis (R.O.)-purified water to appropriate concentrations.

[U-ring-$^{14}$C]atrazine (97% radiochemical purity; sp. act. 14.8 x $10^7$ Bq $mmol^{-1}$) was provided by Novartis Canada Ltd. (Mississauga, ON). The following analytical standards (> 97% purity) were obtained from Ciba-Geigy Co. (Greensboro, NC): atrazine, hydroxyatrazine, deethylatrazine, deisopropylatrazine, deethyldeisopropylatrazine, deethyl-hydroxyatrazine,deisopropylhydroxyatrazine and deethyldeisopropylhydroxyatrazine.

### *2.3. Analytical methods*

Concentrations of MeOH-extractable atrazine were determined by HPLC with a UV detector (Waters 486 Tunable Absorbance Detector, Waters Corporation, Milford, Massachusetts) and the following operating conditions: column: reversed-phase, 15.0 cm x 3.9 mm (5 ism spherical particle C18 packing); mobile phase:

acetonitrile-water (40:60) at 0.9 ml $min^{-1}$; detector wavelength: 220 nm.

In some incubations, radiolabelled material was used to determine the mass balance and chemical identity of herbicide transformation products. Soil microcosm and column incubations were done in containers as described below. Herbicide mineralization was determined by trapping $CO_2$ in a scintillation vial containing 5 ml of 1 N NaOH. Periodically the trap was removed and replaced. Tests established that the quantity of $CO_2$ produced during the experiment was insufficient to neutralize the NaOH and render the trap ineffective. Non-extractable herbicide residues were determined by combusting soil samples using a Model OX 300 biological oxidizer (R.J. Harvey Instrument Corp., Hillsdale, NJ). The 0.5 g samples (duplicates) were mixed with cellulose (1:1) to optimize recovery. Samples were combusted for 4 min and $^{14}CO_2$ was trapped in Carbon-14 Scintillation Cocktail (R.J. Harvey Instrument Corp., Hillsdale, NJ) using plastic collection vials. Recovery efficiency of the oxidizer was found to be 97%. Radioactivity in methanol extracts, trapping solution and NaOH traps was determined by Liquid Scintillation Counting (LSC) using 10 ml of UniverSol Scintillation Cocktail (ICN, Costa Mesa, CA) and correcting for quenching with an external standard. Radioactive atrazine and metabolites in extracts were also quantified by HPLC with a UV detector (Waters 490 Programmable Multiwavelength Detector, Waters Chromatography Division, Milford, MA) and a radioactivity detector (Berthold Model LB506 C-1, Berthold Instruments, Pittsburgh, PA) coupled in series. Instrument operating conditions were as follows: column: reversed-phase, 25.0 cm x 4.6 mm (10 µm Partisil 10 ODS-3 packing); mobile phase: methanol-10 mM ammonium acetate (50:50) at 1 ml $min^{-1}$; detector wavelength: 220 nm. The fraction of $^{14}C$ in parent compound and in metabolites was quantified by integrating peak areas obtained with the radioactivity detector (RD). The presumptive identity of metabolites was established by comparing retention times with those of authentic standards taking the UV-RD offset time into account. At the end of incubations, recovered earthworms were lyophilyzed and combusted and the recovered radioactivity counted.

In the soil microcosm experiments, herbicides were extracted from soil as described in Topp *et al.* (1994) and MeOH-extracted soil was used to quantify the non-extractable residue pool by combustion. In the soil column experiments, non-extractable and extractable herbicide residues in soil and lining were determined as follows: MeOH (7.5 ml) was added to each 0.25 to 0.5 g sample in small Pyrex® glass flasks, shaken for 30 min at 30°C, after which the soil was allowed to settle for 30 min. The supernatant (5 ml) was pipetted into 15 ml glass centrifuge tubes and the soil extracted with MeOH a second time. Supernatants from both extractions were pooled. Tubes containing 10 ml of methanolic extracts were centrifuged for 30 min at 5900 g and a 5 ml subsample was analysed by LSC to determine MeOH-extractable radioactivity. Total radioactivity in soil matrix was measured by combusting two replicated 0.5 g samples taken from each depth in all columns. The non-extractable residue pool at each depth was estimated by subtracting the amount of extractable radioactivity from the total radioactivity. A larger 5 to 10 g subsample of soil matrix was used to determine soil moisture content (w/ w).

*2.4. Soil microcosms*

The influence of earthworms on atrazine dissipation was determined in microcosms consisting of 100 g soil incubated in 250 ml glass pots placed into sealed 1-L mason jars. Treatments were designed to distinguish between the effects of earthworm casts, earthworm-mediated incorporation of herbicide -containing corn residues and earthworm activity on herbicide dissipation. There were eight treatments (Table 1), each in triplicate. Herbicides were applied in a Primextra® stock solution containing 20 µg atrazine $ml^{-1}$. One of three replicate microcosms of each treatment received 300 µg unlabeled atrazine, plus 64 µg of [U-ring-$^{14}C$]atrazine (44 x $10^3$ Bq). The other two replicates received 364 µg unlabeled atrazine.

Microcosms were established with 100 g field moist soil (13% w/w), 1 g corn leaves and Primextra® stock solution (15 ml). Herbicides were either mixed into the soil plus corn leaves, or into the earthworm castings, or surface applied onto the corn leaves (Table 1). For the earthworm cast treatment, one mature *L. terrestris* (4 to 5 g wet weight) in each of three flasks was incubated for 6 weeks with 1 g corn leaves added onto the soil surface; earthworms were then removed, soil containing castings was dried at room temperature to 13% moisture (w/w) and herbicides were mixed in conditioned soil. The amount of food supplied was based on Shipitalo *et al.* (1988), who found that *L. terrestris* in a silt loam soil consumed a maximum amount of 6 mg of corn leaves $g^{-1}$ live worm weight $d^{-1}$, under controlled environmental conditions similar to our earthworm incubations.

Soil was incubated at 12 and 30°C and sampled periodically as described in Table 1. For herbicide dissipation studies at low soil temperatures, flasks in mason jars were incubated at 12°C for 46 d (NOr, WOr, PRr-treatments, Table 1). For the herbicide dissipation studies at high soil temperatures, flasks in mason jars were first incubated at 12°C for 22 d and then at 30°C for 46 d (NOi, WOi, PRi, NOs, WOs-treatments, Table 1). The higher temperature was to

Table 1
Design and sampling days for microcosm experiments

| Code[a] | Treatment combinations | | | Sampling days after herbicide application | Remarks for the first 22 d of incubation at 12°C |
|---|---|---|---|---|---|
| | Soil | Herbicide application | Temperature | | |
| NOr | soil | mix | 12°C | 1, 4, 10, 22, 46 | mixed repeatedly for sampling |
| WOr | soil and worm | mix | 12°C | 1, 4, 10, 22, 46 | mixed repeatedly for sampling |
| PRr | conditioned | mix | 12°C | 1, 4, 10, 22, 46 | mixed repeatedly for sampling |
| NOi | soil | mix | 12°C for 22 d, then 30°C | 22, 23, 26, 32, 44, 68 | undisturbed for 22 d |
| WOi | soil and worm | mix | 12°C for 22 d, then 30°C | 22, 23, 26, 32, 44, 68 | undisturbed for 22 d |
| PRi | conditioned | mix | 12°C for 22 d, then 30°C | 22, 23, 26, 32, 44, 68 | undisturbed for 22 d |
| NOs | soil | surface | 12°C for 22 d, then 30°C | 22, 23, 26, 32, 44, 68 | undisturbed for 22 d |
| WOs | soil and worm | surface | 12°C for 22 d, then 30°C | 22, 23, 26, 32, 44, 68 | undisturbed for 22 d |

[a] NO = no earthworms in soil, WO = earthworms in soil; PR = soil was conditioned with earthworms prior to herbicide applications; r = soil microcosm was mixed repeatedly for sampling at 1, 4, 10 and 22 d after herbicide applications; i = soil microcosm was mixed once to apply herbicides but then left undisturbed at 12°C prior to sampling on d 22; s = herbicides were surface applied and microcosms were left undisturbed at 12°C prior to sampling on d 22.

examine whether earthworm feeding on herbicide-sprayed corn leaves had an subsequent effect on herbicide fate processes in soil after earthworm removal. Temperatures of 30°C, or above, may occur in the surficial layer of field soils, especially in the summer months. Herbicide fate processes are often enhanced at greater soil temperatures, in particular mineralization (Radosevich *et al.*, 1996).

### *2.5. Soil column incubations*

The effects of earthworms on atrazine dissipation and distribution in the soil profile was examined in packed soil column incubations. Tests had established that herbicides did not sorb to the acrylonitrile butadiene styrene (ABS) plastic used to construct the columns and that containers used to incubate columns were gas tight so that recovery of $^{14}CO_2$ would be quantitative. A series of columns (24 cm high, 6.3 cm diameter) were constructed by joining six, 4 cm high ABS rings together with duct-tape. Field-moist (13%), sieved soil ( < 3 mm) was brought to 25% soil moisture content (w/w), re-sieved ( < 3 mm) and sufficient soil was added in increments to obtain a soil bulk density of 1300 kg $m^{-3}$ to a depth of 20 cm. A stainless steel mesh (0.5 mm gauge) was attached to the bottom of each column to prevent earthworm egress.

All columns were conditioned for 4 months at 12°C with a single mature *L. terrestris* (4 to 5 g). One g of corn leaf residues was placed onto the soil surface every 2 weeks to supply food. Columns were covered with plastic sheets and soil moisture losses (determined by weight) were restored once every 2 weeks by adding water onto the soil surface. After 4 months food and water were discontinued to force earthworms to move deeper in the soil. After an additional 2 months, the worms had migrated to the base of the column and were removed. The small amounts of 'loose' surface castings that were egested by the earthworms during conditioning were also removed. Surface castings that were incorporated by the earthworm into the matrix soil of the surficial layer were left in columns. Columns were then saturated from below with a 5 mM $CaSO_4$ solution and drained freely for 4 d to reach field capacity. Earthworm burrows were very stable and did not collapse during the saturation procedure. The number of macropores at both the soil surface and the base of columns was used as a measure to divide columns into two groups with five replicates each. The total number of biopores in soil columns was similar for both groups, on average 4.8 and 1.2 burrow openings at the soil surface and base of the columns, respectively. Subsequently, one group of soil columns received one *L. terrestris* (4 to 5 g) per column; no earthworms were introduced to soil columns of the second group.

Corn leaf residues (1 g) were placed on the soil surface of all columns prior to herbicide applications. Primextra® stock application (6 mL) containing 94 µg atrazine and 113 µg of [U-ring-$^{14}$C]atrazine (77 x $10^3$ Bq) was applied uniformly onto the soil surface using a pipette. Soil columns were incubated at 12°C in containers with NaOH traps for $CO_2$ for 68 d. The watering regime (every 2 weeks) was discontinued. At the conclusion of the experiment, columns were sectioned by removing the duct tape and separating the 4 cm cores to obtain 6 sample depths (0 to 0.5, 0.5 to 4, 4 to 8, 8 to 12, 12 to 16 and 16 to 20 cm). Earthworm burrow linings (1 to 2 mm thickness) were dissected from soil matrix using a spatula, providing 1 to 5 g of material from each depth, depending on the number of burrows. The remaining soil was mixed and subsampled at different locations within the mixture to obtain representative soil matrix samples. It was not possible to distinguish between castings in burrows developed prior to or after herbicide applications. Burrows were also found adjacent to the column wall and at these locations column walls were continuously lined (1 to 2 mm) with material egested by earthworms. This material along the column wall was not included in the representative samples. The small amounts of 'loose' surface castings that were egested by earthworms during the experiment were disregarded. Surface castings that were incorporated by the earthworm into the matrix soil of the surficial layer were considered part of the matrix soil.

*2.6. Data analysis*

The dissipation rate for the herbicides and degradation products was defined as the rate at which the MeOH-extractable parent compound decreases (Fig. 1). Observed dissipation, based on MeOH extractability, is a combination of degradation and the formation of non-extractable residues in the soil. It is distinct from degradation alone in that tightly sorbed or sequestered residues which are not methanol-extractable may subsequently partition back into the extractable phase. Atrazine dissipation

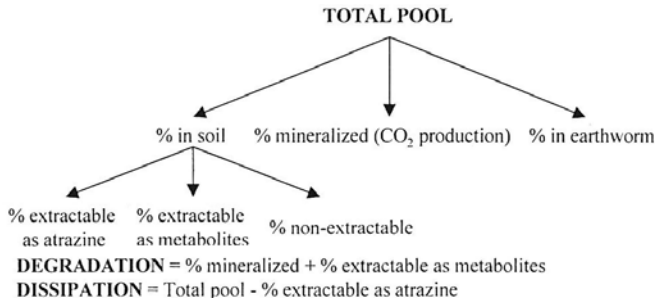


Fig. 1. Flowchart outlining the pools in both microcosms and columns.

rate constants at 12°C and 30°C were calculated for each treatment in triplicate using TableCurve® Windows Version 1.10 (Jandel Scientific). Dissipation rate constants were calculated by nonlinear regression using the assumption that herbicide dissipation was first order:

$$C = C_O\, e^{-kt} \qquad (1)$$

where C = atrazine concentration in soil at time *t* [µg $g^{-1}$]; $C_O$ = atrazine concentration in soil at *t* = 0 [µg $g^{-1}$]; *k* = first order dissipation rate constant [$d^{-1}$]; and *t* = incubation time [d]. For microcosms incubated for 46 d at 12°C (NOr, WOr, PRr, Table 1), initial herbicide concentration in the soil ($C_O$) was calculated by dividing the amount of atrazine in the 15 ml application solution applied by the amount of soil at day zero. For microcosms incubated for 22 d at 12°C and then for 46 d at 30°C (NOi, WOi, PRi, NOs, WOs, Table 1), the amount of extractable atrazine left in the soil after the incubation at 12°C was used for $C_O$. Half-life values were calculated by dividing ln(2) by dissipation rate constants.

The effect of earthworm activity on atrazine half-lives in soil was analyzed using analysis of variance and multiple comparison (Student- Newman-KeulsTest, $P < 0.05$) in SigmaStat® Windows Version 1.0 (Jandel Scientific). For the soil column experiments, the effect of earthworm activity on atrazine distribution and non-extractable residue formation in soil was analysed using the Student-t test ($P < 0.05$) in SigmaStat® Windows Version 1.0 (Jandel Scientific). All statements of significance in this study are at the $P < 0.05$ level.

## *3.* Results

*3.1. Atrazine dissipation in soil microcosms*

The coefficient of determination ($r^2$) of the non-linear regression of atrazine concentration vs. time ranged from 0.88 to 0.99, indicating that the assumption that dissipation rates were first order was appropriate (Table 2). Mass balances were reasonable with recovery of radioactivity ranging from 88 to 103% (12°C) and from 84 to 100% (30°C) for all samples, respectively. Radioactivity in earthworm tissue at 22 and 46 d after herbicide applications was 13 to 18% of that initially applied.

Degradation was the most important pathways for atrazine dissipation at 30°C. After a 68-d incubation, 68 to 92% of the initially applied [U-ring-$^{14}$C]atrazine was mineralized, depending on soil treatment. Treatments at 12°C also showed relatively high atrazine mineralization rates, but only after a lag phase of at least 22 d following herbicide applications. Total mineralization ranged

Table 2. Atrazine dissipation rates and half-life values in soils.
Explanation of treatment codes, incubation temperatures and sampling days are given in Table 1.

| Treatment | Dissipation rate($^{-1}$) | Half-life (d) | Coefficient of determination ($r^2$) |
|---|---|---|---|
| *Treatments at* 12°C [a] | | | |
| NOr | 0.0295 a[b] | 23.6 a | 0.88 |
| WOr | 0.0422 b | 16.5 b | 0.94 |
| PRr | 0.0363 c | 19.1 c | 0.9 |
| *Treatments at both 12 and* 30°C | | | |
| NOi | 0.3145 a | 2.2 a | 0.98 |
| WOi | 0.2725 b | 2.5 b | 0.99 |
| PRi | 0.3275 a | 2.1 a | 0.96 |
| NOs | 0.2849 b | 2.4 b | 0.96 |
| WOs | 0.2360 c | 2.9 c | 0.99 |

[a] Dissipation rates were calculated over the full 46 d of incubation at 12°C.
[b] Means followed by same letters are not significantly different ($P \leq 0.05$).
[C] Treatments were incubated for 22 d at 12°C, then for 46 d at 30°C. Dissipation rates were calculated over the full 46 d of incubation at 30°C. The initial herbicide concentration in the soil was the extractable atrazine in soil at 22 d following incubations at 12°C.

from 36 to 60% of initially applied [U-ring-$^{14}$C]atrazine after 46 d at 12°C, again depending on soil treatment with various hydroxy- and dealkylated metabolites being detected.

Earthworms added to soil after herbicide application significantly shortened the atrazine half-life value by approximately one-third compared to the control without earthworms at 12°C (Table 2). Its half-life value was also significantly less in soil which had been inhabitated by earthworms prior to herbicide application (19 d) when compared to control soil (24 d).

The formation of non-extractable radioactivity in soil was significantly greater in the presence of earthworms. After 22 d at 12°C, non-extractable atrazine residues in the three treatments with earthworms ranged from 18 to 22% of the initially applied radioactivity, compared with 14 to 16% for the three treatments without earthworms (Fig. 2). Although the nature of the non-extractable residues was not identified, hydroxyatrazine (retention time = 7.2 min) was the predominant MeOH-extractable metabolite in all soils, with a minor formation of deethylhydroxyatrazine (retention time = 6.9 min). Following 46 d of incubation, soils with earthworms exhibited a significantly greater percentage of hydroxyatrazine than soils without earthworms, 65 and 41% of the MeOH-extractable fraction, respectively.

After 22-d incubations at 12°C, atrazine mineralization was less than 4% of initial atrazine applied and differences among soil treatments U-ring-14C]atrazine. However, the increased formation of non-extractable atrazine residues in soil, in the presence of earthworms, decreased mineralization amounts after 22 d. After 46 d at 12°C, mineralization was less in soils with *L. terrestris* compared with soils free of *L. terrestris,* at 36 and 53% of the initially-applied [U-ring-$^{14}$C]atrazine,U-ring-$^{14}$C]atrazine, respectively. Soils which contained earthworms for 22 d at 12°C showed significantly smaller atrazine mineralization rates during subsequent incubations at 30°C, compared with soils not incubated with earthworms after herbicide applications, averaging 69 and 85% of the initially applied [U-ring-$^{14}$C]atrazine, respectively. Consequently, the half-life of atrazine at 30°C was greater in soils that contained earthworms following herbicide application compared with other soil treatments (Table 2).

In soil with or without earthworms following herbicide application, the formation of non-extractable atrazine was significantly greater on corn leaves than in surrounding soil. For example, following a 10 d incubation at 12°C, corn leaf samples from soil microcosms contained on average 200 Bq g$^{-1}$ dry matter, compared with 50 Bq g$^{-1}$ dry matter in surrounding soil. In the

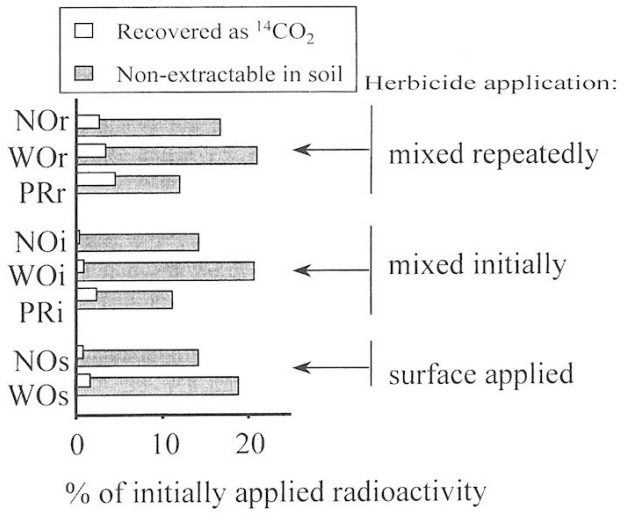


Fig. 2. Atrazine mineralization and non-extractable atrazine residues in soil microcosms at 22 d following herbicide applications. Incubation temperature was 12°C. Explanation of treatment codes are given in Table 1.

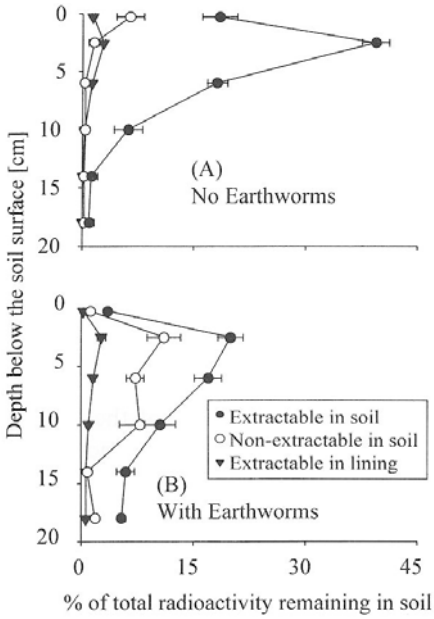


Fig. 3. Distribution of radioactivity at 68 d following herbicide applications on corn leaf residues at the soil surface in soil columns with or without earthworm activity. Symbols are means of five replicates and error bars represent standard error.

conditioned soil, however, corn leaf residues were decomposed by the earthworms prior to herbicide application and less non-extractable atrazine formed. Consequently, [U-ring-$^{14}$C]atrazine was more readily mineralized in the earthworm-conditioned soil than in the other soil treatments, at both 12 and 30°C, thereby producing a smaller half-life at 30°C in the conditioned soil than in the other soil treatments (Table 2).

*3.2. Distribution and dissipation of atrazine in packed columns*

Although earthworm activity stimulated the formation of non-extractable atrazine in soil microcosms, this effect was more pronounced in the soil column experiments (Fig. 3). In soils with earthworms, 30.3% of the initially-applied [U-ring-$^{14}$C]atrazine became non-extractable 68 d after herbicide application, while only 9.3% became non-extractable in columns without earthworms. Total MeOH-extractable radioactivity in soil was significantly greater in columns without *L. terrestris* (84.5%) compared with those with *L. terrestris* (62.6%), expressed as a percentage of the initially-applied [U-ring-$^{14}$C]atrazine.

Total atrazine mineralization in soil was < 3% of initially applied radioactivity whether for treatments with or without earthworms (Fig. 4). Evolved $^{14}CO_2$ was slightly greater in columns with *L. terrestris* compared with columns without *L. terrestris* up to 30 d, after which $^{14}CO_2$ rates increased more rapidly in soil without earthworms. At the conclusion of the experiment, between 93 and 102% of the initially-applied radioactivity was recovered from each of the columns. Radioactivity in earthworm tissue 68 d after herbicide applications was < 8% of that initially applied.

After 68 d, more than 65% of the radioactivity in soils without earthworms was in the top 4 cm layer; only 8% of this material was non-extractable (Fig. 3A). The 0.5 cm surficial soil layer consisted mostly of corn residues, soil and 'aged castings' (excreted by earthworms during the conditioning period to allow for burrow development). The large organic content of the surface soil layer enhanced the formation of non-extractable atrazine. The amount of non-extractable radioactivity was 4 times greater in the top 0.5 cm than in the 0.5 to 4 cm layer and at least 15 times greater than at any other depth. The percentage of MeOH-extractable residues was significantly greater in the 0.5 to 4 cm soil layer (39.4%) than in the top 0.5 cm soil layer (18.5%) due to dispersion and diffusion processes throughout the 68 d experiment. It was unlikely that any measurable radioactivity moved below 0.5 cm during the herbicide applications since only 6 ml of herbicide solution was added at a slow rate (1 drop $s^{-1}$). At the end of the experiment, no radioactivity was detected below 12 cm in three out of five columns, suggesting little movement of atrazine residues by dispersion or diffusion and confirming adsorption onto soil colloids and corn residues at the soil surface (Fig.5A, C and E).

Radioactivity was more evenly redistributed in the soil profile of columns with earthworms compared to those without earthworms. The percentage of total soil radioactivity in soils with earthworms was least in the top 0.5 cm soil layer (Fig. 3B). Corn residues were detected only below the top 0.5 cm soil layer, suggesting herbicide transport from the surface by *L. terrestris* feeding

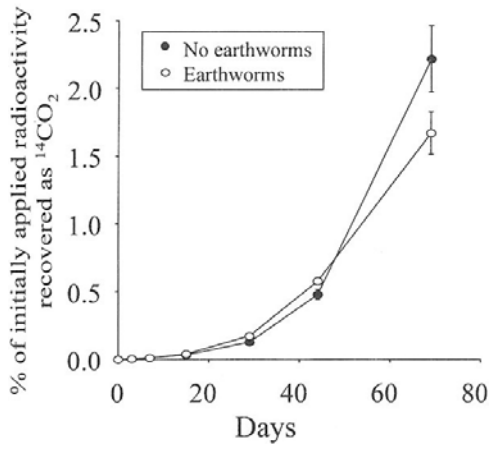


Fig. 4. Atrazine mineralization rates in soil columns with or without earthworm activity. Symbols are means of five replicates and error bars represent standard error.

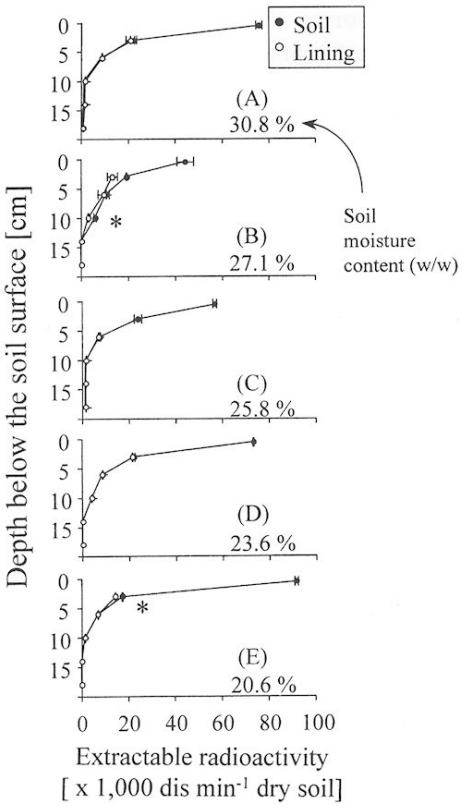


Fig. 5. Distribution and concentration of extractable radioactivity in lining and matrix soil at 68 d following herbicide applications on corn leaf residues at the soil surface in soil columns with biopores and without earthworm activity. Circles are means of two to four replicates and error bars represent standard error. Asterisks indicate concentration of non- extractable radioactivity in matrix soil is significantly greater than that in lining material. A, B, C, D and E are each one of the five column replicates. The soil moisture content was measured at the end of the experiment and each value is an average of all layers in the column combined.

or burying activity on surficial crop residues. Herbicide was transported by earthworm activity throughout the entire soil column, but the extent varied among replicates (Fig. 6). The radioactivity varied from 6 to 18% (expressed as a percentage of the total radioactivity in the soil) in the 12 to 16 cm layer and 6 to 9% in the 16 to 20 cm layers.

Earthworms ingested corn residues with sorbed atrazine residues on the soil surface and subsequently egested much of these materials, mostly in their burrows, as castings. In columns with earthworms, there was significantly more extractable radioactivity observed in burrow linings than in matrix material at similar depths (Fig. 5). Generally, no significant differences in extractable radioactivity were observed between burrow linings and soil matrix in the columns with biopores containing no earthworms during the experiment (Fig. 5).

Earthworms increased the amount of non-extractable radioactivity throughout the soil profile (Fig. 3). Total non-extractable residues in soils with earthworms were greater in the 0.5 to 12 cm soil layer (25%) compared to the 0 to 0.5 cm (1.3%) and 12 to 20 cm (1.9%) layers, expressed as a percentage of total radioactivity remaining in the soil. Earthworms may have been most active from the 0.5 to 12 cm depth, resulting in a greater concentration of non-extractable radioactivity.

## 4. Discussion

The high rate of mineralization of the s-triazine ring at 30°C incubations and preponderance of hydroxy metabolites in the extractable fraction indicated that microbial atrazine hydrolysis to hydroxyatrazine was the most important pathway of atrazine degradation in this loamy soil. Atrazine half-life values ranged from 2 to 3 d at 30°C and were within the range of that reported for similar

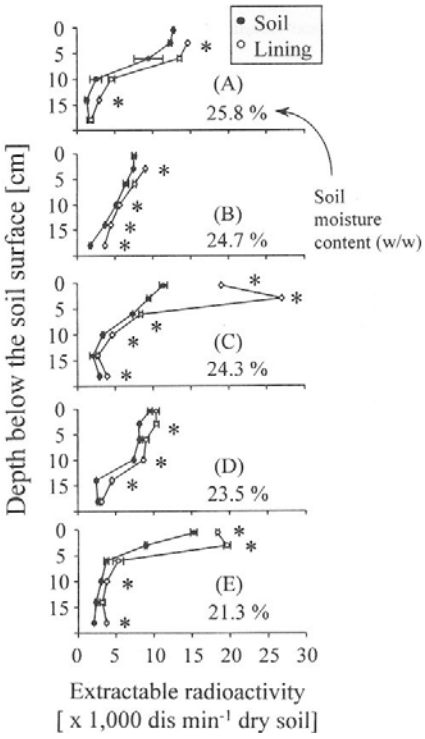


Fig. 6. Distribution and concentration of extractable radioactivity in lining and matrix soil at 68 d following herbicide applications on corn leaf residues at the soil surface in soil columns with biopores and with earthworm activity. Circles are means of two to four replicates and error bars represent standard error. Asterisks indicate concentration of non-extractable radioactivity in matrix soil is significantly greater than that in lining material. A, B, C, D and E are each one of the five column replicates. The soil moisture content was measured at the end of the experiment and each value is an average of all layers in the column combined.

temperatures in enrichment cultures of atrazine-mineralizing bacteria and soils inoculated with atrazine-degrading bacteria (Mandelbaum *et al.*, 1993; Assaf and Turco, 1994) and in agricultural soils containing active atrazine-degrading microbial communities (Topp *et al.*, 1996; Vanderheyden et al., 1997). Atrazine mineralization rates in soil columns were less than in soil microcosms at 12°C. There were several possible explanations, including the experimental design. Soil columns were left undisturbed for 68 d, while soil microcosms were mixed for sampling and this may have increased the bioavailability of atrazine to mineralizing microorganisms.

While atrazine mineralization rates were greatest in soils which had been processed by earthworms (creating castings), the direct influence of earthworm activity stimulated sorption of atrazine and metabolites on soil, such that these residues were more likely to be bound by soil than completely mineralized. Radosevich *et al.* (1996) also found that sorption limited atrazine mineralization at 30°C. Herbicide residues strongly bound to soil are less available to microorganisms and thus have lesser biodegradation and mineralization potential than those in soil solution or those less strongly sorbed (Khan and Ivarson, 1981; Scribner *et al.*, 1992).

Earthworms were able to increase herbicide sorption in soil due to (a) physical processes, such as the burying and mixing of herbicide-sprayed crop residues into soil and (b) feeding activity, resulting in the ingestion of herbicide-sprayed crop residues and mixing these with soil in their digestive tract. The exact mechanisms by which earthworms increase the formation of non-extractable atrazine residues in soil are difficult to identify. Possible hypotheses were that earthworms enhanced the physical trapping of atrazine residues on clay and humic structures and the absorption of atrazine residues into the network of organic matter (partitioning). For example, the ingestion and mixing of [U-ring-$^{14}$C]atrazine residues with mineral and organic substances in the earthworm digestive tract could have increased the sorption of herbicides by soil. Shipitalo and Protz (1988) reported that *L. terrestris* feeding activity influenced bonding between clay and organic matter in soil. It is also possible that the earthworm gut contained a large and active atrazine-degrading microbial community accelerating the transformation of atrazine into hydroxyatrazine which is more strongly sorbed than atrazine by soil (Clay and Koskinen, 1990). Hydroxyatrazine is more likely to be adsorbed onto organic matter than atrazine because the replacement of the chlorine atom by a hydroxyl group permits additional hydrogen bonding (Moreau and Mouvet, 1997). Metabolites such as hydroxyatrazine and other hydroxy derivatives readily form non-extractable residues in soils (Khan and Saidak, 1981; Erickson and Lee, 1989). Previous studies have found that the gut content of *L. terrestris* contained greater microbial populations than the soil matrix (Parle, 1963; Barois, 1992; Trigo and Lavelle, 1993). Chio and Sanborg (1978) showed that *L. terrestris* was able to transform atrazine into several degradation products within 48 h after injections of atrazine into the haemocoel of the earthworm.

The translocation of organic material from the soil surface to depth by earthworms has been reported by Darwin (1881); Edwards and Heath (1962); Shipitalo *et al.* (1988). Gallagher and Wollenhaupt (1997) found that a *L. terrestris* population of 40 to 60 per m$^2$ in an alfalfa no-till field incorporated 26% of surface residues into the soil during 30 d in the spring. We additionally found that earthworms can rapidly move herbicides deeper into the soil. This phenomenon may have widespread significance to atrazine persistence in the field, in particular in agricultural areas where atrazine is frequently used and earthworms numbers in soil are great. For example, in southern Ontario there is about $10^6$ ha in corn that may use about 2 kg atrazine ha$^{-1}$ (2 million kg annually). There can be as many as 50 adult *L. terrestris* earthworms m$^{-2}$ in soils under corn, soybeans or wheat in Ontario (de St. Remy and Daynard, 1982; Tomlin *et al.*, 1995) resulting in an estimated 5 x $10^{11}$ earthworms for southern Ontario's cultivated soils. That number would translate to 2.5 x $10^9$ kg of fresh weight earthworm biomass that is very likely to influence herbicide fate in these agricultural fields. Even so, atrazine movement by earthworms in field soils would be normally less we observed in this study. When the herbicide is applied in the field not all of the chemical is intercepted by crop residues and only part of the crop residues on the surface in no-till is incorporated by earthworms into the soil. The effects of earthworms on herbicides would depend on the herbicide formulation (e.g. granular products, wettable powder), its application method (foliar applied, soil applied) and timing (pre-plant, preemergence, postemergence). *L. terrestris is* most active in spring to early summer (when herbicides are commonly applied) and in fall. The removal of surface crop residues to depth by earthworms needs to be monitored under field conditions in these seasons, especially with respect to how this process affects weed control and herbicide persistence.

Based on the herbicide concentration in burrow linings versus matrix soil in our study, we expect that castings in field soils, whether deposited on the surface or at depth, would have a greater atrazine concentration, when compared to the matrix soil. We found small amounts of surface castings in the columns and most castings were egested by earthworms at depth (burrow linings). In field soils, the ratio surface-to-sub-

surface castings may be different and *L. terrestris* also forms middens.

Earthworm burrows can function as preferential flow paths which may rapidly transport atrazine to depth. Preferential herbicide transport occurs when rainfall exceeds the infiltration capacity of the soil and when herbicides are available on or near the soil surface. We showed that earthworms transported herbicide-sprayed crop residues from the soil surface to depth. Earthworm feeding on atrazine-sprayed crop residues at the soil surface decreased the potential for atrazine transport into preferential flow paths such as biopores. Because earthworms increased the amount of non-extractable atrazine residues in the soil, the potential for herbicide transport through the soil profile would be accordingly reduced since strongly sorbed herbicides are less likely to leach.

## 5. Conclusions

Earthworms added to soil after herbicide application significantly shortened the atrazine half-life by one-third, compared with earthworm-free soils. The greatest effect of earthworms on the fate of atrazine in soil was to accelerate the formation of soil-bound atrazine residues. In incubations with earthworm castings (but no earthworms), non-extractable atrazine residues were formed to a lesser extent and atrazine was more readily mineralized. Earthworms transported atrazine from the soil surface to depth by feeding on herbicide-sprayed crop residues. After 68 d, earthworms had translocated almost two-third of the surface applied [U-ring-$^{14}$C]atrazine below 4 cm soil depth, depositing twice as much in their burrow as in surrounding matrix soil. In soils containing no earthworms, more than 65% of the initially applied [U-ring-$^{14}$C]atrazine remained in the top 4 cm.

Earthworm feeding activity may significantly influence atrazine dissipation processes in agricultural soils as well as transporting herbicides down the soil profile. Atrazine mineralization rates tend to be greater in soils which have been processed by earthworms (as castings), but the direct influence of earthworm activity stimulates sorption of atrazine and metabolites on soil, such that these residues are more likely to be bound than completely mineralized.

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