Influence of the Laboratory Incubation Method on Chlorotoluron and Terbutryn Degradation in Soil

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Degradation of the herbicides chlorotoluron and terbutryn was assayed using three different incubation systems: columns of undisturbed soil, columns packed with sieved soil, and flasks. Two different soils were employed, a sandy loam (A) and a loam soil (B), and different temperature (4−32 °C) and moisture content (7−13%) conditions were assayed. Dissipation of both herbicides in soil A for treatment T2 (25 °C and 10% of moisture content) was significantly different in the three different incubation systems assayed. In the case of soil B, with lower organic matter content, the obtained half-lives for treatment T2 were statistically indistinguishable in all the incubation systems, with the exception of the flask incubation of terbutryn. Incubation in packed cores generally yielded longer half-lives than flask incubations in all the other treatments for both soils and both compounds. Differences among incubation systems were greater with terbutryn than with chlorotoluron, which has a lower sorption coefficient.

Keywords: *Degradation; chlorotoluron; terbutryn; incubation method; soil*

INTRODUCTION

Chlorotoluron and terbutryn are employed in binary mixtures for pre- or postemergence selective control of grasses and broad-leaved weeds in winter cereal crops. Although these herbicides are widely used, only a few studies have been published on the persistence of these compounds in soil (Smith and Briggs, 1978; Avidov et al., 1985; Rüdel et al., 1993). Degradation is one of the most important processes that determine the fate of herbicides in soil, especially in cereal crops without irrigation. In these conditions, other pathways for herbicide dissipation, like leaching, are of relatively lesser importance. The obtention of reliable degradation parameters is thus a key step in order to estimate or predict, using mathematical simulation models, the persistence of these herbicides in soil.

Different authors follow a variety of approaches to determine the dissipation of herbicides in soil. Numerous experiments using field plots treated with herbicide have been carried out (Gross et al., 1979; Avidov, 1985; Walker and Brown, 1985) to assess field dissipation rates. Lysimeter studies with undisturbed soil cores placed outdoors have also been accomplished (Leake, 1991; Kördel et al., 1991; Rüdel et al., 1993) as a more realistic means to determine herbicide fate than laboratory experiments without the complications and uncertainties of field studies. Laboratory incubation systems are also diverse, including biometer systems consisting of glass dishes with sieved soil (Rüdel et al., 1993; Skidmore et al., 1994); incubation of sieved and air-dried soil in glass vials (Graham-Bryce et al., 1982), in polypropylene bottles (Walker and Brown, 1985; Walker et al., 1989), or in flasks (Zimdahl et al., 1970; Smith and Briggs, 1978; Topp et al., 1994); and also incubations in cores of undisturbed soil or in columns packed with sieved soil (Topp et al., 1994). The effects of temperature and water content in the obtained degradation rates have been studied in depth (Hurle and Walker, 1980; Walker, 1978), but the effects of other variables, such as the disruption of structure caused by sieving, the air-drying of the soil, the application rate, volume, and solvent, changes in the pesticide availability and extractability, and the different distribution of the chemical in the soil in the various incubation systems employed, may also be important. There is little published information on the effects of different incubation systems on the degradation rate. Rüdel et al. (1993) compared the degradation of two herbicides under laboratory and outdoor conditions and observed not only the influence of temperature on the degradation but also the effect of the disruption of soil structure caused by sieving. Topp et al. (1994) found that there were no significant differences in the dissipation rates of atrazine and metolachlor incubated in flasks and intact cores, although the dissipation rates of these herbicides were significantly slower in packed cores.

The aim of the work reported here was (i) to obtain the degradation rates of chlorotoluron and terbutryn in two different soils, under different conditions of temperature and moisture content, and (ii) to compare the degradation rates obtained with three different incubation systems: columns of undisturbed soil, columns packed with sieved soil, and flasks.

Table 1. Physical and Chemical Properties of the Soils

soil	texture class	clay (%)	sand (%)	organic matter (%)	pH	soil classification
A	sandy loam	11.54	64.81	1.75	6.68	Xerofluvent
B	loam	18.22	44.34	0.97	7.69	Haploxeralf

MATERIALS AND METHODS

Soils and Herbicides. Two different soils were used in this study, a sandy loam and a loam soil whose physical and chemical properties are given in Table 1. Organic matter content was determined by oxidation with potassium dichromate, pH was measured using a glass electrode pH meter in a mixture of soil and distilled water (1:2.5 by volume), and the texture was determined using the pipet method (MAPA, 1994).

Soil for incubation experiments in flasks and packed cores was collected from the upper layer (0−10 cm) of the fields, air-dried at room temperature, and passed through a 2-mm mesh

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Table 2. Incubation Conditions for Degradation Experiments

treatment	temp (°C)	soil moisture content (%)	incubation systems
T1	4	10	flask
			packed core
T2	25	10	flask
			packed core
			intact core
T3	32	10	flask
			packed core
T4	25	7	flask
			packed core
T5	25	13	flask
			packed core

sieve. Soil for incubation in intact cores was collected undisturbed with PVC cores, 10 cm length × 6 cm i.d. Insertion produced no visible compression of the soil. Both ends of the columns were capped with plastic lids. Twelve replicates of each soil were collected and air-dried at room temperature.

The herbicide used was a formulation containing as active ingredients chlorotoluron [3-(3-chloro-*p*-tolyl)-1,1-dimethylurea] and terbutryn [N^2-*tert*-butyl-N^4-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine]. The commercial formulation used was Dicuran Extra, Ciba-Geigy, with 43 g of chlorotoluron and 7 g of terbutryn in 100 cm^3 of the formulation, applied at a rate of 8 L/ha.

Analytical Techniques. Determination of herbicide levels in soil was carried out by using a procedure based on a previously published method for phenylureas (Pérez et al., 1991). A brief description of the procedure follows: Soil (20 g) was extracted twice with methanol (100 mL) on an orbital shaker for 45 min. The extract was filtered and the filter cake washed with methanol (2 × 25 mL). The solvent was evaporated to dryness and the residue dissolved in ethyl acetate/hexane (6:1 by volume). An aliquot was analyzed by a Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen−phosphorus detector and automatic injector. A capillary column HP-1 (12 m × 0.22 mm i.d.) was employed. Helium was used as a carrier gas at 1 mL/min. The column was held at 70 °C for 1 min and then programmed at 15 °C/min to 230 °C and held for 1 min.

Incubation Systems. Soil was incubated in the laboratory during 60−70 days in three different systems: flasks, undisturbed cores, and packed cores. Comparison of the three incubation systems was performed at 25 °C and a soil moisture content of 10% ($g/g_{soil\ dry\ wt}$). Further incubation conditions were assayed with flasks and packed cores. These conditions are summarized in Table 2.

Flask Incubation. Soil for incubation experiments in flasks was treated by spreading the soil in a plastic tray and applying an aqueous suspension of the commercial formulation of the herbicides to yield a final herbicide concentration around 2.5 μg/g of chlorotoluron and 0.4 μg/g of terbutryn similar of that found in soil treated with 8 L/ha of Dicuran (Lechón, 1996). After treatment, the soil was thoroughly mixed by being passed twice trough the 2-mm mesh sieve and was allowed to equilibrate for 24 h at 4 °C. A subsample of 20 g of each soil was taken to determine the soil moisture content. After the equilibration period, samples (300 g) of the treated soil were transferred to screw-top glass jars, and the appropriate amount of water was added to give the required soil moisture content of each treatment. Treatments were replicated twice. Samples (20 g) were taken for herbicide analysis 1 day after addition of herbicide and then every 10−15 days. Water contents were adjusted on each sampling date.

Packed Cores Incubation. PVC cores (10 cm length by 6 cm i.d.) with PVC caps in the lower end were packed with air-dried and sieved soil (300 g). The surface of the soil of each column was evenly pipetted with 2 mL of an aqueous suspension of the commercial formulation of the herbicides at a rate of 8 L/ha. After treatment the cores were covered with a plastic cap and allowed to equilibrate for 24 h at 4 °C. Two replicate cores per soil type and sampling date were prepared. After the equilibration period, two cores were collected, and the soil from each core was removed and thoroughly sieved four times. Two samples (20 g) from each core were taken for herbicide analysis, and one further sample was taken to determine the moisture content. Water was added to each core to give the required soil moisture content of each treatment, and this moisture content was maintained constant by adding the appropriate amount of water each sampling date. Two replicate columns were collected every 15 days, and the soil was handled as described for the initial sampling.

Table 3. Degradation Constants (*k*) and Half-Lives (*H*) of Chlorotoluron in Soil A with Different Incubation Methods

treatment	method	k (±SE) (day^{-1})	H^a (days)	r^2
T1	packed core	0.01059 ± 0.00133	65 a	0.8416
	flask	0.02994 ± 0.00098	23 b	0.9760
T2	packed core	0.02397 ± 0.00124	29 a	0.9492
	flask	0.03860 ± 0.00228	18 b	0.9522
	intact core	0.03250 ± 0.00182	21 c	0.9522
T3	packed core	0.03032 ± 0.00167	23 a	0.9430
	flask	0.05647 ± 0.0239	12 b	0.9622
T4	packed core	0.02633 ± 0.00150	26 a	0.9393
	flask	0.03076 ± 0.00270	23 a	0.8500
T5	packed core	0.03798 ± 0.00163	18 a	0.9646
	flask	0.06842 ± 0.00325	10 b	0.9547

[a] Half-lives followed by different letters are significantly different at the 0.025 level within each treatment.

Intact Core Incubation. Undisturbed cores were collected from each soil as described previously and allowed to air-dry by removal of the upper lid. Two cores were taken to determine the moisture content gravimetrically and ensure that it was below the 10%. The surface of the soil of each column was treated with the herbicide solution as described previously for packed cores, and the cores were covered and allowed to equilibrate for 24 h at 4 °C. The day after herbicide application, two replicate cores were taken to determine herbicide and moisture content, following the same procedure as that for packed cores. The appropriate amount of water was then added to each column to reach a moisture content of 10%, which was maintained constant along the incubation assay. Two replicate cores were taken on each sampling date (every 15 days). The moisture content was checked for each core and found to be close to 10% (9.5 ± 0.9).

Data Analysis. Data from each degradation experiment were fitted to a first-order law by linear regression of the log of concentration vs time to get the first-order degradation rates and coefficients of determination. Statistically significant differences among these degradation rates were established by subjecting the data to an ANOVA test using the general linear models procedure of the STATISTIX package, Version 1.1.

RESULTS AND DISCUSSION

Herbicide degradation constants and half-lives obtained in both soils in the different treatments and incubation systems are shown in Tables 3 and 4 for chlorotoluron and in Tables 5 and 6 for terbutryn. The values of the determination coefficient (r^2) were always significative at the 1% level, meaning that the kinetics of the dissipation closely fit the first-order law. Degradation is quicker at higher temperature or moisture content. The chlorotoluron degradation rate increases nearly 2 times due to the temperature or moisture increase in the range studied. The effect of moisture content on the rate increase of terbutryn degradation is similar, while the effect of temperature is higher, increasing the degradation rate 2.4 and 6.9 times for soils A and B, respectively, in flasks and 9.7 and 15.7 times in packed cores, in the range studied. Soil A showed a higher degradation capacity than soil B, likely due to the higher organic matter content of soil A and the consequent higher microbial activity (Kaufman and Kearney, 1970), considering that the process of degradation of these herbicides is mainly microbial (Geiss-

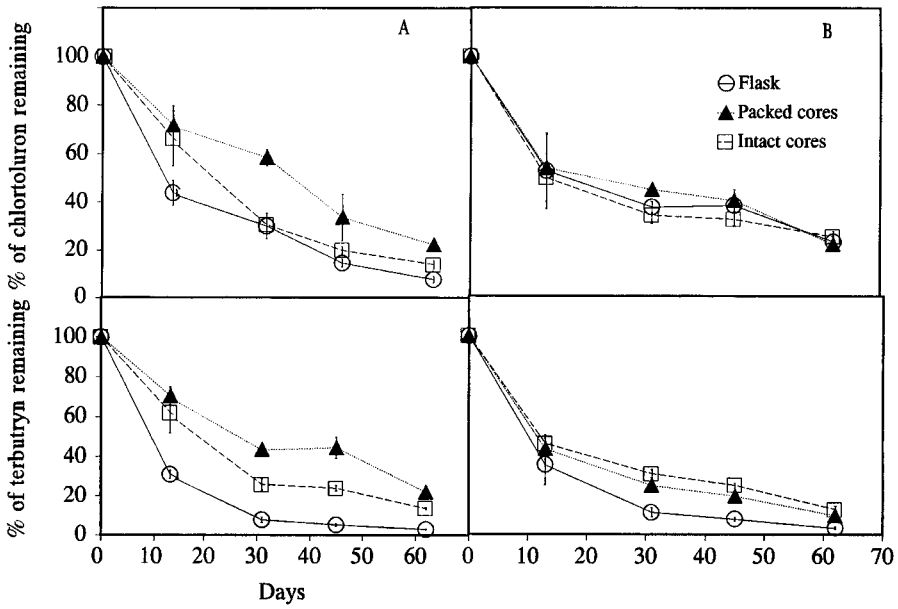

Figure 1. Dissipation of chlorotoluron and terbutryn in soils A and B incubated at 25 °C and 10% moisture content (treatment T2) using three incubation systems: flasks, packed cores, and intact cores. Vertical bars show standard deviations of replicates.

Table 4. Degradation Constants (*k*) and Half-Lives (*H*) of Chlorotoluron in Soil B with Different Incubation Methods

treatment	method	k (±SE) (day^{-1})	H^a (days)	r^2
T1	packed core	0.00943 ± 0.00075	74 a	0.9076
	flask	0.01643 ± 0.00179	42 b	0.8669
T2	packed core	0.02173 ± 0.00179	32 a	0.8802
	flask	0.01935 ± 0.00265	36 a	0.7698
	intact core	0.01865 ± 0.00237	37 a	0.7449
T3	packed core	0.02609 ± 0.00298	27 a	0.7934
	flask	0.03095 ± 0.0368	22 a	0.8871
T4	packed core	0.01582 ± 0.00161	44 a	0.8282
	flask	0.01777 ± 0.00186	39 a	0.8833
T5	packed core	0.02849 ± 0.00167	24 a	0.9358
	flask	0.05233 ± 0.00515	13 b	0.9197

[a] Half-lives followed by different letters are significantly different at the 0.025 level within each treatment.

Table 5. Degradation Constants (*k*) and Half-Lives (*H*) of Terbutryn in Soil A with Different Incubation Methods

treatment	method	k (±SE) (day^{-1})	H^a (days)	r^2
T1	packed core	0.00431 ± 0.00098	161 a	0.6185
	flask	0.04111 ± 0.00454	17 b	0.7888
T2	packed core	0.02260 ± 0.00132	31 a	0.9358
	flask	0.05364 ± 0.00377	13 b	0.9310
	intact core	0.03091 ± 0.00216	22 c	0.9275
T3	packed core	0.04166 ± 0.00180	17 a	0.9641
	flask	0.09844 ± 0.0579	7 b	0.9665
T4	packed core	0.01935 ± 0.00133	36 a	0.9133
	flask	0.07622 ± 0.00656	9 b	0.8544
T5	packed core	0.04086 ± 0.00127	17 a	0.9809
	flask	0.07982 ± 0.00941	9 b	0.8001

[a] Half-lives followed by different letters are significantly different at the 0.025 level within each treatment.

bühler et al., 1975; Kaufman and Kearney, 1970; Avidov et al., 1985).

Reported laboratory half-lives for chlorotoluron in the scientific literature vary from 28 to 56 days at 25 °C (Smith and Briggs, 1978) and from 40 to 93 days at 20 °C (Rüdel et al., 1993). Values obtained by Smith and Briggs (1978) are in agreement with our findings, while values reported by Rüdel et al. (1993) are somewhat longer. As far as terbutryn is concerned, the available reported laboratory half-life is 14 days at 26 °C (Avidov et al., 1985), which is in agreement with our results with flask incubation. There is a wide range of reported terbutryn half-lives in the field varying from 7 to 358 days. Our results also show this wide range of degradation rates depending on the incubation conditions and system.

Table 6. Degradation Constants (*k*) and Half-Lives (*H*) of Terbutryn in Soil B with Different Incubation Methods

treatment	method	k (±SE) (day^{-1})	H^a (days)	r^2
T1	packed core	0.00305 ± 0.00041	227 a	0.7759
	flask	0.01186 ± 0.00134	58 b	0.8577
T2	packed core	0.03644 ± 0.00156	19 a	0.9646
	flask	0.05316 ± 0.00296	13 b	0.9526
	intact core	0.03010 ± 0.00168	23 a	0.9524
T3	packed core	0.04782 ± 0.00335	14 a	0.9108
	flask	0.08236 ± 0.0625	8 b	0.9508
T4	packed core	0.02247 ± 0.00118	31 a	0.9478
	flask	0.03653 ± 0.00406	19 b	0.9001
T5	packed core	0.05015 ± 0.00271	14 a	0.9448
	flask	0.08827 ± 0.00669	8 b	0.9509

[a] Half-lives followed by different letters are significantly different at the 0.025 level within each treatment.

Figure 1 depicts the kinetics of the decline in chlorotoluron and terbutryn contents with time in both soils and the three incubation systems for the treatment conducted at 25 °C and 10% water content (T2). Dissipation of both chlorotoluron and terbutryn in soil A was significantly different ($P < 0.025$) in the three different incubation systems assayed (Tables 3 and 5). Incubation in flasks yielded the shortest half-lives always for terbutryn and in most treatments with soil A for chlorotoluron, and incubation in packed cores the longest half-lives. The shorter half-life obtained in incubation in flasks can be explained by the increased pesticide−soil contact produced by the treatment of the whole mass of soil and the careful mixing obtained by sieving four times. This increased contact would enhance biodegradation by favoring the availability of the pesticide to the microorganisms. This positive effect would counteract the inhibitive effect that the sieving and air-drying of the soil could have on the activity of microbial populations. Moreover, this increased contact would diminish the herbicide extractability during the course of the incubation, due to the formation of bound residues. In contrast, in the incubation in packed cores the herbicide was applied at the surface and the dispersion of the compound in the soil was limited to the additions of water to maintain the moisture content.

Most of the herbicide would probably remain in a thin layer of the soil near the surface and be unavailable for the microorganisms present in the rest of the soil. Similar results were reported by Skidmore et al. (1994), who also observed that an improved distribution of the chemical in the soil leed to an increase in the rate of permethrin mineralization. In addition, soil microflora could be negatively affected by the handling of the soil (air-drying and sieving). Furthermore, the packing process of the column would probably lead to a higher bulk density of the soil, compared to the density of both the intact cores, which conserve the natural macrostructure of the soil, and the flasks, which are subjected to periodical stirring in the sampling process. The increased bulk density would diminish aeration and therefore would slow biodegradation (Kaufman and Kearney, 1970). These three factors could account for the degradation shown by this incubation system being the slowest compared to that shown by the other two methods. Incubation in undisturbed cores would present an intermediate behavior since the distribution of the chemical is also restricted to the upper layer of the column, but it lacks the disadvantages of the soil-handling process and maintains the macrostructure of the soil. Similar results were obtained by Topp et al. (1994) with the degradation of atrazine and metolachlor. Although these researchers did not find significant differences among intact cores and flasks, packed cores always showed the slowest degradation rate.

Half-lives obtained in treatment T2 (25 °C and 10% moisture content) for both compounds in soil B (Tables 4 and 6) were statistically indistinguishable in all of the incubation systems, with the exception of the flask incubation of terbutryn in soil B, which gave a shorter half-life than the two other systems.

Incubation in packed cores yielded longer half-lives than flask incubations in all of the other treatments for both soils and both compounds although the observed differences were not always statistically significant. Soil B always showed less marked differences among incubation systems than soil A for both chlorotoluron and terbutryn. The obtained rate of chlorotoluron degradation in soil B (Table 4) was statistically indistinguishable between flasks and packed cores for all of the treatments with the exception of the treatments with the highest and lowest half-lives, T1 (4 °C and 10% moisture content) and T5 (25 °C and 13% moisture content), respectively. In general, the treatments with extreme temperature or moisture content conditions, as treatments T1 at 4 °C, T3 at 32 °C, and T5 at 13% moisture content, generally produced significant differences. When differences among incubation systems appeared, they were always greater with terbutryn than with chlorotoluron. The higher adsorption coefficient of terbutryn, $K_{OC} = 393$, in comparison with chlorotoluron, $K_{OC} = 109$ (Lechón, 1996), could explain this different behavior, since adsorption would reduce the bioavailability of the pesticide to degrading microorganisms and also would limit transport of the pesticide to other layers of the soil column (Scribner et al., 1992).

In summary, our results indicate that the incubation method can have an influence on the degradation of herbicides in soil. Dissipation of chlorotoluron and terbutryn was significantly different in the three incubation systems when assayed with one soil, while differences were always less marked with the other soil having a lower organic matter content. Incubation in packed cores generally yielded longer half-lives than flask incubations, and differences among incubation systems were greater with tebutryn than with chlorotoluron, which has a lower adsorption constant.

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