Performance of slow-release formulations of alachlor

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ABSTRACT

Alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide] is a widely used herbicide that has been identified as a groundwater contaminant. Several slow-release formulations (SRFs) of this herbicide have been examined for their capacity to reduce its leaching and enhance weed control. A field experiment was performed to determine alachlor leaching and bioefficacy in: (i) a commercial formulation, Alanex, (ii) SRFs based on ethylcellulose (EC) microencapsulation or phosphatidylcholine (PC)-montmorillonite complexes. Alachlor distribution within the top 30 cm of the soil layer was modeled with the pesticide-leaching model PEARL, using release constants for the SRFs obtained from *in-vitro* experiments. Compared to the commercial formulation, leaching to the 20- to 30 -cm depth was reduced by 33% for EC and 25% for PC-clay formulations, but only in the latter was herbicide bioefficacy retained. The poor bioefficacy of the EC formulations can be explained by their very slow release properties, which may nevertheless confer an advantage under prolonged heavy rainfall. The model yielded good predictions for the residual amounts of herbicide at 59 and 99 d after treatment (DAT). At 191 DAT, the amounts of alachlor at 0- to 20 -cm depth were overestimated by 13 to 23%, indicating limitations of the model at longer times, attributed to: (i) the adaptation time of the microbial population for herbicide degradation and (ii) the effect of herbicide sequestration in the soil matrix, a phenomenon denoted as “aging”.

1 **Abbreviations:** SRF: slow-release formulation; EC: ethylcellulose; PC:
2 phosphatidylcholine; *a.i.*: active ingredient; RMSE: root mean square error; DAT: days
3 after treatment; AEW: acetone/ethyl acetate/water

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INTRODUCTION

Herbicide mobility in soils causes: (i) groundwater contamination, (ii) reduced herbicidal activity and crop yield due to herbicide leaching below the weed seed zone, and (iii) surface migration of the herbicide which can harm neighboring crops (Weber et al., 1999). SRFs of herbicides are designed to maintain the threshold concentration of the active ingredient for weed control in the soil by providing release at the required rate. The consequent lowering of the required amounts of herbicide is both environmentally friendly and economically advantageous.

Ethylcellulose (EC)-based formulations of the herbicides norflurazon and alachlor have been obtained by the solvent-evaporation technique (Fernández-Urrusuno et al., 2000; Sopeña et al., 2005). The release rate of the active ingredient from EC-based formulations depends on the pesticide-to-EC ratio and is strongly correlated with the size of the microcapsule (Pérez-Martínez et al., 2001; Morillo et al., 2004). In soils, with limited alachlor persistence, the use of EC-based formulations of the herbicide reduced its loss by 54% relative to the use of a commercial formulation (Sopeña et al., 2008). Similarly, the leaching of alachlor from the EC formulations was significantly reduced in soil column experiments over the commercial formulation (Sopeña et al., 2007).

A new approach for the development of SRFs of herbicides consists of their incorporation in micelles or vesicles of alkylammonium quaternary cations adsorbed on clay minerals (Nir et al., 2006). An optimal vesicle-clay formulation of anionic herbicides, in which a large fraction of the herbicide is adsorbed by the vesicle/micelle-clay complex, can be obtained for particular vesicle (or micelle)-clay ratios, such that most of the vesicles or micelles are adsorbed without undergoing premature decomposition (Mishael et al., 2002a; Undabeytia et al., 2004). Clay-vesicle (micelle)

formulations of sulfonylurea herbicides have been shown to reduce the amount of leached herbicide several-fold and enhanced its biological activity in the upper soil layer (Mishaal et al., 2002b, 2003; Undabeytia et al., 2003). These formulations provide weed control at much lower rates than with commercial formulations. Recently, surfactants were replaced with the EPA-approved substance phosphatidylcholine (PC) (Sánchez-Verdejo et al., 2008a). PC-clay formulations of atrazine and alachlor have enabled reducing the doses of these herbicides to below the recommended values (Sánchez-Verdejo et al., 2008b).

In the current study, the performance of SRFs of the herbicide alachlor using EC-based microcapsules and PC-clay complexes was compared, the criteria being their ability to reduce leaching while maintaining herbicide activity. Such studies are scarce, and alachlor was selected because it is a widely used herbicide in the United States (Boparai et al., 2006) and Europe (Sánchez-Camazano et al., 2005).

The use of mathematical models for predicting the environmental fate of pesticides is becoming increasingly common. Pesticide-leaching models can be divided into chromatographic flow models, which are based on the convection/dispersion equation, and preferential flow models (Scorza et al., 2005). The Pesticide Emission Assessment at Regional and Local scales model (PEARL model) (Tiktak et al., 2000) is one of the chromatographic models that has been adopted by the European Union in their pesticide-regulation scheme.

The objectives of this work were: (i) to evaluate the performance of SRFs of the herbicide alachlor that use two different methodologies to reduce leaching and maintain herbicide efficacy in field experiments in comparison to a commercial formulation, and (ii) to test the validity of the PEARL model in simulating alachlor leaching.

MATERIALS AND METHODS

Field experiments

A field experiment was conducted to test and compare the efficiency of the PC- and EC-based alachlor formulations. The experiment was carried out in a sandy loam soil classified as a Typic Calcixerept (Soil Survey Staff, 2006) located at “La Hampa” in Coria del Río (Seville, SW Spain) (6°5’10’’ W, 37°17’15’’ N). The soil contained 49 g kg⁻¹ of CaCO₃, 13.6 g kg⁻¹ of organic matter and its pH was 8.9. The particle-size distribution was: 131 g kg⁻¹ clay, 68 g kg⁻¹ silt, and 801 g kg⁻¹ sand. The study treatments were a commercial formulation (Alanex), two EC (A14 and A18), and two PC-clay (A5/8 and A1.6/14) formulations. The commercial formulation was applied as a stable emulsion, and the SRFs were applied as aqueous suspensions. All of the formulations were sprayed on November 21, 2006, and left on the soil surface. The applied rate was 3 kg ha⁻¹.

A completely randomized experimental design (three replicates per treatment) was conducted in 18 subplots (1 m x 1 m). A commercial formulation, two PC-clay, and two ethylcellulose based-formulations were tested versus a control. Oat (*Avena sativa* L.) (30 g m⁻²) was sown in all plots. At selected times after herbicide application (59, 99, and 191 d), soil samples of each plot were taken from different soil depths (0 to -10, 10 to -20 and 20 to -30 cm) using a small auger probe. No irrigation was applied. The plots received 46, 158, and 247 mm of rain before the first, second, and third sampling, respectively.

The amount of alachlor at each depth was extracted in triplicate using 2 g of soil and 10 mL of methanol, and equilibrated for 24 h under continuous shaking. The suspensions were centrifuged and the supernatant was analyzed for the herbicide concentration. Preliminary experiments with non-treated soil spiked with aliquots of

herbicide solutions showed full recovery of the herbicide. At the end of the experiment on May 30, 2007, the plant shoots were harvested and the bioefficacy of alachlor was evaluated by weighing the fresh shoots.

Preparation of alachlor-PC-clay formulations

The PC formulations of alachlor were prepared by dissolving different amounts of the herbicide in a 6 mM PC solution by sonication, and further addition to montmorillonite, which sorbs PC-alachlor complexes. The added concentrations of herbicides were 8.0 and 14.0 mM for 5 and 1.6 g L⁻¹ clay, respectively. After shaking for 24 h, the suspensions were centrifuged at 12100 g for 20 min. The supernatants were analyzed for the remaining herbicide and the pellets were freeze-dried to yield the final formulations. Additional details of the preparation are found in Sánchez-Verdejo et al. (2008a). A nomenclature was introduced for PC-clay formulations (Table 1) where the first letter (A) indicates alachlor, the first number denotes the clay concentration and the second number gives the initial herbicide concentration used. The active ingredient (*a.i.*) was determined for each formulation by measuring the herbicide concentration in solution by HPLC after sonicating 5 mg of the formulation with 10 mL of methanol in duplicate.

Preparation of the alachlor-ethylcellulose (EC) formulations.

The ethylcellulose-alachlor formulations were prepared by the oil-in-water emulsion solvent evaporation technique, using polyvinyl alcohol (PVA) as the emulsifier and two types of EC with different viscosities (EC10 and EC40) as the matrix polymer (Sopeña et al., 2005). Briefly, 1 g of polymer and 0.2 or 0.3 g of herbicide were dissolved in 15 mL of chloroform at room temperature. The herbicide-

polymer solution was then emulsified into an aqueous phase by dropwise addition into 150 mL of aqueous solution containing 0.15% of PVA while stirring at either 600 or 300 rpm. Two formulations (A14 and A18) were prepared using different experimental conditions. The A14 formulation used EC40, a 3.33:1 EC/A ratio, and a stirring speed of 600 rpm. A18 was prepared by using EC10, a 5:1 EC/A ratio, a stirring speed of 300 rpm, and polyethylene glycol (PEG) as a channel forming agent, which was added to the polymer solution (40% w:w) before the formation of the emulsion with the aqueous phase. The active ingredient was determined by measuring the herbicide concentration by HPLC after dissolving 5 mg of each formulation in methanol in duplicate.

Water release kinetics

Release tests of alachlor formulations were performed in duplicate with a rotating paddle apparatus (Sotax). For each formulation, 5 mg of alachlor were added to 1 L of deionized water at 25°C and stirred at 50 rpm. At appropriate time intervals from 0 to 96 h, samples were taken, passed through PTFE filters, and the herbicide was analyzed. Alachlor was analyzed by HPLC (Shimadzu Model 10A) equipped with a PDA detector. The reverse phase column was a 15 cm Kromasil 100 C18, and the flow rate was 1.0 mL min⁻¹. The mobile phase was 60% acetonitrile and 40% water. The wavelength was set at 220 nm, and the retention time was 6.26 min.

Modeling of leaching

The chromatographic flow model PEARL (version 3.3.3) was used for calculating the distribution of alachlor into the soil for the applied formulations. Soil hydraulic properties were introduced by using the retentivity-conductivity relationships of Van Genuchten (1980) and Mualem (1976). Pesticide physicochemical parameters,

such as molecular weight, vapor pressure, and water solubility were taken from the literature (Tomlin, 2003). Additional input parameters were the sorption coefficient normalized to the organic matter content (K_{om}) and the degradation half-life time in soil, which were determined from adsorption and incubation experiments in non-treated soil, respectively. The cropping scenario was winter cereal oats, with an alachlor application rate of 3 kg of *a.i.* ha⁻¹. Mean daily values of temperature and rainfall were taken from the meteorological station located on the farm.

Application of the model to slow release formulations was used as suggested by Ford et al. (2007) based on the optional use of a subroutine to include the degradation of the herbicide to its metabolites. The release of the active substance from the formulation is simulated simply as the degradation of parent to metabolite ($t_{1/2}(2)$) and the K_{om} of the parent was set to the maximum value required to prevent movement through the soil profile. The resulting metabolite, which is in fact the active substance, was modeled for degradation using the experimentally determined $t_{1/2}$ data ($t_{1/2}(1)$). In the current work, the new modeling approach involves the use of the half-life time determined from the water release kinetics experiments as $t_{1/2}(2)$.

Adsorption and dissipation experiments

Non-treated soil adjacent to the field experiment was sampled for incubation and adsorption experiments. Incubation experiments were done in triplicate by adding alachlor (3 mg kg⁻¹ soil) to 200 g of air-dried soil. After shaking thoroughly for 24 h, the soil was transferred to plastic pots, covered with aluminum foil and incubated in the dark for 2 weeks at 20°C. The soil moisture content was maintained at field capacity by the periodic addition of water. Soil samples (2 g) were taken every two or three consecutive days, extracted with methanol, and the herbicide content was determined.

Adsorption experiments were done by equilibrating 2 g of untreated soil with 10 mL of alachlor solutions of concentrations up to 20 mg L⁻¹ for 24 h at 20°C. The supernatants were centrifuged and analyzed for the herbicide. The amount of adsorbed alachlor was calculated from the difference between the initial and equilibrium solution concentrations. The experimental points were fitted to the Freundlich equation ($\text{Conc.}_{\text{sorbed}} = K_f \text{Conc.}_{\text{water}}^{1/n}$) and K_{om} was successively determined from the value of the sorption coefficient K_f normalized to the soil organic matter content.

Data analysis

A one-way ANOVA was used to analyze differences in crop yield among the different formulations. The means were compared with the Student's t-test ($\alpha=0.05$), using SAS software (SAS Institute, 2008). The residual amounts of herbicide from each depth studied were analyzed using the standard error as a pooled estimate.

RESULTS

Active ingredient and encapsulation efficiency

Table 1 shows the characteristics of the formulations used in the field experiments. The active ingredient (*a.i.*) contents in the PC-clay formulations were 15.0% and 39.5% for the A5/8 and A1.6/14 formulations, respectively. The *a.i.* content of the A5/8 formulation was quite close to that of the A18 formulation. The *a.i.* content of the A1.6/14 formulation was similar to the *a.i.* content of the commercial formulation (48%).

The encapsulation efficacy (EE) of the herbicide was defined as the ratio between the amount of alachlor that was incorporated into the microspheres or the PC-clay complex and the amount added to the system. The PC-clay complex A1.6/14 was

the best system for incorporating the herbicide, which is of economical interest in the preparation of herbicide formulations.

Water release kinetics

Figure 1 shows the cumulative release of alachlor from PC-clay and EC formulations as well as the solubility profile for technical grade alachlor. The release pattern from the PC-clay formulations (A5/8 and A1.6/14) was similar during the first 7 h. In both cases, the initial release was fast, reaching complete release at the end of the experiment. The release from EC formulations was significantly slower and complete release was not measurable.

The kinetics of alachlor release from the PC-clay and EC formulations was analyzed by fitting the data to the generalized model of Ritger and Peppas (1987)

$$M_t/M_o = K t^n \quad [\text{Eq. 1}]$$

where M_t is the amount of herbicide released from the formulations at any time t , M_o is the total initial amount of herbicide in the system, K is a constant that incorporates the characteristics of the macromolecular network system, and n is an exponent that indicates the mechanism of release. It should be noted that this expression is valid for a limited time span since it cannot be extended to infinite values of t . Ignoring a residual adsorbed fraction of alachlor at equilibrium is permissible because the concentrations of the formulations were very small.

According to the correlation coefficients, the experimental data were well-fitted by the Ritger and Peppas equation (Table 2). The exponential n values in Eq. 1 were about 0.48 and 0.55 for the PC-clay complexes, whereas lower values of 0.42 and 0.43 were obtained for the EC formulations. It has been reported that in cases of pure Fickian release, the exponent n has the limiting value of 0.50 and 0.43 for release from slabs and

spheres, respectively (Ritger and Peppas, 1987). Consequently, the mechanism of alachlor release from EC and PC-clay formulations is close to a Fickian controlled diffusion process. This mechanism has been already reported for norflurazon EC microspheres (Sopeña et al., 2005).

The $t_{1/2}$ values (the times at which 50% of the herbicide is released) were deduced from the K and n constants (Table 2). These $t_{1/2}$ values are those defined as $t_{1/2}$ (2) in the modeling scheme. The $t_{1/2}$ values were extremely high for EC formulations. For example, the $t_{1/2}$ values for the two formulations containing practically the same *a.i.* content, A18 and A5/8, were 51-fold larger for the EC formulations. The capability of using these $t_{1/2}$ values in the model arises from the fact that the release curves were also well-fitted to a first order kinetics equation (data not shown), and therefore the release times were independent of the herbicide concentration used in the *in-vitro* experiments.

Alachlor distribution in the soil profile

The extracted amounts of alachlor in the soil core columns after the three samplings are shown in Fig. 2 and Table 3. The total amount of alachlor remaining in the 0- to 30 -cm depth was similar for the first and second samplings for the commercial and PC-clay formulations. The percentages of the total alachlor applied remaining in the soil after 59 d were 76.7 ± 6.4 for the commercial formulation, 82.5 ± 7.7 for A5/8, 82.9 ± 4.6 for A1.6/14, 87.1 ± 18.0 for A14 and 100.1 ± 7.3 for A18. After 247 mm of rain (third sampling, 191 DAT), the retained amounts of alachlor for the commercial and PC-clay formulations were still similar: 56.7 ± 3.0 , 55.5 ± 2.5 and $52.9 \pm 6.2\%$ for the commercial, A5/8, and A1.6/14, respectively. The EC formulations exhibited, however, much higher recoveries, $74.5 \pm 10.7\%$ for A14 and $98.7 \pm 7.9\%$ for A18.

Fig. 2a shows that for the first soil sampling, which corresponded to 46 mm of cumulative rainfall, the amount of alachlor was larger in the 0- to 10 -cm depth for the PC-clay and EC formulations than for the commercial formulation. The increase of alachlor amounts with respect to the commercial formulation was 28.8% for A5/8, 36.6% for A1.6/14, 42.7% for A14, and 100% for A18. In the next layer, there was a larger accumulation of the herbicide from the commercial formulation than from the SRFs. At 99 d and 112 mm of cumulative rainfall (Fig. 2b), the amount of alachlor in the 0- to 10 -cm soil layer was reduced for the commercial and PC-clay formulations. There was still, however, a significant difference in the top layer soil between the commercial formulation and SRFs.

At 191 d and 247 mm of cumulative rainfall (Fig. 2c), the recovered amounts from the 0- to 10 -cm depth were significantly larger for the SRFs than for the commercial formulation. The accumulated amounts of alachlor at the 20- to 30 -cm depth were much larger for the commercial formulation than for SRFs. The lower recovery of the herbicide from PC-clay and EC formulations at the 20- to 30 -cm depth and the greater recovery at the 0- to 10 -cm depth imply that the use of these formulations reduces leaching of alachlor over the commercial formulation.

The bioefficacy of the commercial and slow release formulations at the end of the field experiment was determined (Table 4). The reductions in fresh weight with respect to the control were 48.3%, 52.4%, and 57.2% for the commercial, A5/8 and A1.6/14 formulations, respectively, but no significant difference in the yield of the crop was observed. In contrast, EC formulations did not lead to any reduction in the fresh weight, as their yield was comparable to the control.

Modeling alachlor distribution in the soil

The experimental and calculated distribution of alachlor is shown in Table 3. In this analysis, values of K_{om} and degradation half-life time of 2.13 L kg^{-1} and 13 days, as determined previously from adsorption and incubation experiments in non-treated soil, were used. The release half-lives determined in the water release kinetics experiment (Table 2) were used to model the SRFs. In general, the predicted amounts agreed quite well with the measured amounts 59 and 99 days after treatment (DAT) for the commercial and the slow release formulations (RMSE=6.3% and 4.2%, respectively). In the upper layer of soil, the residual amounts of herbicide from the commercial formulation were always overestimated. In PC-clay formulations, the experimental values were slightly underestimated for the model at 59 DAT and overestimated at 99 DAT, but the overall agreement with the predicted values was very good. In contrast, the opposite trend was seen for the EC formulations, whose calculated values were overestimated after 59 DAT and underestimated after 99 DAT. There was very good agreement between the predictions of the model and the experimental amounts for A14 at 99 DAT. For all formulations, there was generally good agreement between the experimental and predicted amounts at the lower depths.

The predictions of the herbicide distribution in the soil were not adequate at 191 DAT (RMSE=12.5%). The differences in the upper layer became more pronounced, especially for the commercial and PC-clay formulations, whose calculated amounts were overestimated by 19-23%. With the EC formulations, the predicted amount was quite close to the experimental amount for A18, but there was no good correspondence between the experimental and predicted amounts for A14. In the following segment, the model largely overestimated the residual amounts in the soil for the commercial and PC-clay formulations by 13%, whereas the agreement was better

for EC formulations. At a depth of 20- to 30 -cm, the predictions were quite good with the exception of the commercial formulation.

DISCUSSION

The high recoveries of herbicide for the commercial and all the SRFs showed very slow herbicide dissipation. The high persistence of alachlor may be explained by: (i) the effect of the herbicide concentration on the soil microbial activity; (ii) climatic factors, such as temperature and soil moisture content; and (iii) the effect of drying and wetting cycles on the soil. In the case of SRFs, an additional factor is the protection of the active ingredient through its inclusion in a matrix, such as PC-clay or EC, which slows its release and field dissipation.

Alachlor behavior in soil is influenced by the organic carbon content. Its interaction with organic matter has been reported to occur mainly via physical adsorption processes, such as weak induction or dispersion forces (Patakioutas and Albanis, 2002). There is a strong correlation between its persistence and sorption, which determines the remaining fraction of herbicide that is immediately available for different dissipation mechanisms. The vapor pressure of alachlor is low and losses by volatilization and photolysis in the soil are not significant. The main pathway for alachlor dissipation in soils is through microbial degradation (Tomlin, 2003). Sopeña et al. (2008) found a strong correlation between the soil dehydrogenase activity, and alachlor dissipation in a soil similar to that examined in our study. Felsot and Dzanter (1995) reported that alachlor inhibited this enzyme at very low concentrations, and caused prolonged inhibition for at least 21 d at concentrations $\geq 750 \text{ mg kg}^{-1}$. The amounts of herbicide extracted with methanol account for its bioavailable bound residues, which prolong its herbicidal activity on the microbial flora, because of the low

organic carbon content of this soil combined with the weak interactions of the herbicide with the organic matter.

The low rate of alachlor dissipation can also be attributed to the generally low temperatures (Fig. 3). Yen et al. (2005) reported that the half-life of alachlor increased from 68 to 187 d when decreasing the incubation temperature decreased from 25°C to 10°C at a soil moisture content of 90% of the field capacity. The temperatures recorded during our experiment were in the range of 8- to 13 °C range, with the exception of the last month and a half, which had higher values (~17-20°C).

The rainfall (Fig. 3) was very irregular, with intervals of dry periods. García-Valcárcel and Tadeo (1999) reported that drying and rewetting cycles of soil yielded lower degradation rate of the herbicides simazine and hexazinone, compared to soil incubated in the same range of constant soil moisture. Therefore, the effect of alternating drying and wetting periods would give a lower herbicide degradation rate, partly explaining the high persistence of alachlor.

This study demonstrates a reduction in the leaching of alachlor from PC-clay and EC formulations compared to that from the commercial formulation. The reduction in leaching was reflected by the larger extracted amounts of herbicide after 191 DAT in the 0- to 10 -cm layer of the soil together with the lower extracted amounts in the 20- to 30 -cm depth relative to the commercial formulation. In the top soil layer, bioefficacy of the SRFs was maintained only in the PC-clay formulations.

The poor performance of the EC formulations in terms of maintaining the herbicide bioefficacy was due to the low release constant of the herbicide from the matrix. Previous laboratory studies (Sopeña et al. 2007; 2008) have reported that these EC formulations significantly reduced the dissipation and leaching of alachlor. The differences in performance of EC formulations under laboratory vs. field conditions

may be related to the soil moisture. The occurrence of rainfall is a major factor in herbicide release from granular SRFs immediately after application. The granules must be wetted to enable permeation of the active ingredient through the microcapsule shell and its diffusion into the soil solution before it can move through the soil and be available for pest control. The oscillation in soil moisture content, or the effect of drying and wetting cycles is believed to reduce herbicide release and create several lag times in the release profile, producing sub-threshold concentrations of alachlor in the soil matrix. In addition, EC formulations have an average particle size distribution in the range of 100 to 400 μm (Sopeña et al., 2009), which makes the wetting of the granules under low rainfall events more difficult in comparison to PC formulations, which have a particle size of around 2 μm .

The model yielded good predictions at times of 99 DAT. For longer times (191 DAT), a discrepancy was recorded between the experimental and predicted amounts of the herbicide in the top soil layer (Table 3). The model overestimated the concentration of alachlor, which may be partly explained by an enhanced degradation of the herbicide following adaptation of the microbial populations. Scorza and Boesten (2005) reduced the half-life of bentazone by 2.5-fold in order to simulate its leaching. Hence, in our study, calculations were performed to account for this effect by decreasing the half-life of alachlor. The inaccuracy in the calculated values was largely reduced by employing a 1.6-fold reduction in its half-life (8 days) for the period between 99 and 191 DAT. The predicted values in the 0- to 10 -cm depth were 21.5% for the commercial formulation, 26.3% for A5/8, 28.3% for A1.6/14, 31.1% for A14 and 44.1% for A18. This improved fitting was expressed by a much lower RMSE (7.9% versus 18.0%).

Another source of uncertainty in the predictions may arise from herbicide aging in the soil, which the model does not account for: this process does not occur when determining the half-life of alachlor under laboratory conditions due to its relatively rapid degradation. Because of this aging, the fraction of extractable residues decreases at the expense of non-extractable /recalcitrant residues, resulting in a reduction in the bioavailability and degradation of the herbicide (Boivin et al., 2004). This fraction is included in the calculations, but is not susceptible to leaching and degradation processes; therefore, the calculations will yield an overestimation of the residual amounts in the soil, as noted in Table 3. The effect of sequestration of the active compound in the soil is manifested by the difficulty in extracting part of this recalcitrant fraction, which requires increasingly harsh solvents (Taylor et al., 2005). There is a good correlation between bioavailability and the soil fraction extracted with methanol (Kelsey et al., 1997; Barriuso et al., 2004). A sequential extraction after methanol with a mixture of acetone/ethyl acetate/water (AEW) (2:2:1 v:v) will extract some of the recalcitrant fractions (Laabs and Amelung, 2005). This sequential extraction was performed after 191 d for the commercial and the PC-clay formulations, where a longer aging period for the herbicide is expected due to the longer residence time of the free herbicide in the soil. With EC formulations, in which the active ingredient is retained for longer periods within the EC matrix, herbicide aging is less important. The AEW-desorbed fractions in the top soil layer amounted to $15.2 \pm 7.4\%$, $10.5 \pm 0.3\%$, and $10.1 \pm 1.5\%$ for the commercial, A5/8, and A1.6/14 formulations, respectively. These AEW recoveries are indicative of the aging of the herbicide in the soil, but the aged fraction of the herbicide might account for a larger amount than that determined by AEW extraction, since there could be an important fraction of the herbicide incorporated into the soil matrix by abiotic processes.

CONCLUSIONS

This study clearly showed reduced leaching of alachlor from PC-clay and EC formulations as compared to the commercial formulation. In the top soil layer, alachlor's bioefficacy was only maintained with the PC-clay formulation: the very slow release from the EC formulations results in herbicide concentrations in the soil solution that are too low to be effective.

The persistence of the herbicide in field experiments was much longer than that determined in the laboratory under constant temperature and moisture conditions. This can be explained mainly by climatic factors, such as oscillations in temperature and soil moisture content, or by the effect of drying and wetting cycles on the bioavailability of the herbicide in the soil. In the case of SRFs, an additional factor is protection of the active ingredient through its inclusion in a matrix which limits the herbicide's exposure to environmental factors, slowing its dissipation in the field.

Tests of the pesticide-leaching model PEARL demonstrated good prediction for the experimental distribution in the soil profile at 59 and 99 DAT; however, a large discrepancy was noted between the calculated and experimental values at the end of the experiment (191 DAT). The implication may be that the model has to account for two additional effects: (i) the adaptation time of the microbial population for degrading the herbicide and (ii) the effect of herbicide sequestration in the soil matrix by a process denoted as "aging".

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Figure legends.

Figure 1. Cumulative release of alachlor in *in vitro* experiments from the technical product (■), and the slow-release formulations A5/8 (●), A1.6/14 (▲), A18 (▼) and A14 (◆).

Figure 2. Alachlor distribution from commercial and slow-release formulations as a function of soil depth 59 (a), 99 (b) and 191 (c) days after treatment. Means followed by the same letter by each formulation by each depth for each period of sampling are not significantly different according to Student's multiple range test at $P=0.05$. Vertical bars show standard errors.

Figure 3. Fortnightly average precipitation (mm rain) (●) and air temperature (°C) (■) during the field experiment.

Table 1. Characterization of alachlor formulations based on EC and PC-clay in this study.

Formulant material	Notation	Active ingredient (<i>a.i.</i>) content (%)	Encapsulation Efficacy (EE) (%)
PC-clay	A5/8	15.0	78
PC-clay	A1.6/14	39.5	97
Ethylcellulose	A14	20.6	75
Ethylcellulose	A18	13.8	70

Table 2. Parameters deduced from simulations of alachlor release in water by Eq. (1).

Formulation	$K (h)^{-n}$	n	R^2	$t_{1/2}(h)$
A5/8	14.06	0.48	0.992	18
A1.6/14	14.07	0.55	0.995	15
A14	3.52	0.42	0.998	576
A18	2.71	0.43	0.996	912

Table 3. Experimental (Exp.) and calculated (Calc.) distribution of alachlor along the soil profile expressed as percent of the total amount applied, after 59, 99 and 191 days after treatment.†

		Soil depth					
		0-10 cm		10-20 cm		20-30 cm	
		Exp	Calc	Exp	Calc	Exp	Calc
59 DAT	Com	35.8±1.3‡	40.0	30.2±4.2	28.7	10.7±0.9	18.6
	A5/8	46.1±2.8	43.3	22.9±1.4	29.7	13.5±1.4	19.1
	A1.6/14	49.0±1.0	43.2	20.7±2.8	29.7	13.2±0.8	19.3
	A14	51.1±4.3	58.3	19.8±3.8	17.6	16.2±6.9	8.4
	A18	70.0±9.2	79.6	16.8±0.5	12.6	13.3±1.6	6.1
99 DAT	Com	28.2±1.7	33.6	29.0±0.9	27.5	22.1±1.7	17.7
	A5/8	35.1±0.2	41.7	26.9±1.5	28.5	17.9±0.3	18.2
	A1.6/14	37.7±3.9	42.0	23.5±0.5	28.3	19.7±2.1	18.0
	A14	53.2±6.5	52.7	16.8±2.3	20.6	17.7±1.6	13.0
	A18	63.0±5.9	57.3	20.3±0.4	18.2	18.5±0.8	11.5
191 DAT	Com	14.6±1.6	33.4	14.5±0.1	27.3	27.6±1.3	17.8
	A5/8	18.5±1.9	41.5	15.8±0.5	28.2	21.2±0.1	18.1
	A1.6/14	19.0±1.4	41.7	15.1±3.1	28.6	18.8±0.4	18.0
	A14	35.1±6.5	49.5	19.2±5.7	21.7	20.2±6.5	13.8
	A18	54.0±1.9	49.8	30.2±4.4	21.0	14.5±1.6	13.9

†. The root mean square error (RMSE) was 6.3% for 59 DAT, 4.2% for 99 DAT and 12.5% for 191 DAT.

‡. Plus/ minus standard error.

Table 4. Avena yield as affected by alachlor formulation.[†]

Formulation	Avena yield (Mg ha ⁻¹)
None	69.7A
Commercial	36.0B
A5/8	33.1B
A1.6/14	29.8B
A14	59.5A
A18	7.05A

[†]. Means with the same letter are not significantly different at $P=0.05$ as determined by Student's multiple range test.

Figure 1

Figure 2

Figure 3