

Performance of slow-release formulations ofalachlor

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3 **ABSTRACT**

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

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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

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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)

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

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

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

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

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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

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

4 5 **Preparation of alachlor-PC-clay formulations**

6 The PC formulations of alachlor were prepared by dissolving different amounts
7 of the herbicide in a 6 mM PC solution by sonication, and further addition to
8 montmorillonite, which sorbs PC-alachlor complexes. The added concentrations of
9 herbicides were 8.0 and 14.0 mM for 5 and 1.6 g L⁻¹ clay, respectively. After shaking
10 for 24 h, the suspensions were centrifuged at 12100 g for 20 min. The supernatants were
11 analyzed for the remaining herbicide and the pellets were freeze-dried to yield the final
12 formulations. Additional details of the preparation are found in Sánchez-Verdejo et al.
13 (2008a). A nomenclature was introduced for PC-clay formulations (Table 1) where the
14 first letter (A) indicates alachlor, the first number denotes the clay concentration and the
15 second number gives the initial herbicide concentration used. The active ingredient (*a.i.*)
16 was determined for each formulation by measuring the herbicide concentration in
17 solution by HPLC after sonicating 5 mg of the formulation with 10 mL of methanol in
18 duplicate.

19 20 **Preparation of the alachlor-ethylcellulose (EC) formulations.**

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

1 polymer solution was then emulsified into an aqueous phase by dropwise addition into
2 150 mL of aqueous solution containing 0.15% of PVA while stirring at either 600 or
3 300 rpm. Two formulations (A14 and A18) were prepared using different experimental
4 conditions. The A14 formulation used EC40, a 3.33:1 EC/A ratio, and a stirring speed
5 of 600 rpm. A18 was prepared by using EC10, a 5:1 EC/A ratio, a stirring speed of 300
6 rpm, and polyethylene glycol (PEG) as a channel forming agent, which was added to
7 the polymer solution (40% w:w) before the formation of the emulsion with the aqueous
8 phase. The active ingredient was determined by measuring the herbicide concentration
9 by HPLC after dissolving 5 mg of each formulation in methanol in duplicate.

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Water release kinetics

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

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Modeling of leaching

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

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

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

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18 **Adsorption and dissipation experiments**

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

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

8 9 **Data analysis**

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

14 15 **RESULTS**

16 **Active ingredient and encapsulation efficiency**

17 Table 1 shows the characteristics of the formulations used in the field
18 experiments. The active ingredient (*a.i.*) contents in the PC-clay formulations were
19 15.0% and 39.5% for the A5/8 and A1.6/14 formulations, respectively. The *a.i.* content
20 of the A5/8 formulation was quite close to that of the A18 formulation. The *a.i.* content
21 of the A1.6/14 formulation was similar to the *a.i.* content of the commercial formulation
22 (48%).

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

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

4 **Water release kinetics**

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

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

$$13 \quad M_t/M_o = K t^n \quad [\text{Eq. 1}]$$

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

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

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

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

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14 **Alachlor distribution in the soil profile**

15 The extracted amounts of alachlor in the soil core columns after the three
16 samplings are shown in Fig. 2 and Table 3. The total amount of alachlor remaining in
17 the 0- to 30 -cm depth was similar for the first and second samplings for the commercial
18 and PC-clay formulations. The percentages of the total alachlor applied remaining in the
19 soil after 59 d were 76.7 ± 6.4 for the commercial formulation, 82.5 ± 7.7 for A5/8,
20 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
21 (third sampling, 191 DAT), the retained amounts of alachlor for the commercial and
22 PC-clay formulations were still similar: 56.7 ± 3.0 , 55.5 ± 2.5 and $52.9\pm 6.2\%$ for the
23 commercial, A5/8, and A1.6/14, respectively. The EC formulations exhibited, however,
24 much higher recoveries, $74.5\pm 10.7\%$ for A14 and $98.7\pm 7.9\%$ for A18.

1 Fig. 2a shows that for the first soil sampling, which corresponded to 46 mm
2 of cumulative rainfall, the amount of alachlor was larger in the 0- to 10 -cm depth for
3 the PC-clay and EC formulations than for the commercial formulation. The increase of
4 alachlor amounts with respect to the commercial formulation was 28.8% for A5/8,
5 36.6% for A1.6/14, 42.7% for A14, and 100% for A18. In the next layer, there was a
6 larger accumulation of the herbicide from the commercial formulation than from the
7 SRFs. At 99 d and 112 mm of cumulative rainfall (Fig. 2b), the amount of alachlor in
8 the 0- to 10 -cm soil layer was reduced for the commercial and PC-clay formulations.
9 There was still, however, a significant difference in the top layer soil between the
10 commercial formulation and SRFs.

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

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

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Modeling alachlor distribution in the soil

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

17 The predictions of the herbicide distribution in the soil were not adequate at
18 191 DAT (RMSE=12.5%). The differences in the upper layer became more
19 pronounced, especially for the commercial and PC-clay formulations, whose calculated
20 amounts were overestimated by 19-23%. With the EC formulations, the predicted
21 amount was quite close to the experimental amount for A18, but there was no good
22 correspondence between the experimental and predicted amounts for A14. In the
23 following segment, the model largely overestimated the residual amounts in the soil for
24 the commercial and PC-clay formulations by 13%, whereas the agreement was better

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

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DISCUSSION

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

12 Alachlor behavior in soil is influenced by the organic carbon content. Its
13 interaction with organic matter has been reported to occur mainly via physical
14 adsorption processes, such as weak induction or dispersion forces (Patakioutas and
15 Albanis, 2002). There is a strong correlation between its persistence and sorption, which
16 determines the remaining fraction of herbicide that is immediately available for
17 different dissipation mechanisms. The vapor pressure of alachlor is low and losses by
18 volatilization and photolysis in the soil are not significant. The main pathway for
19 alachlor dissipation in soils is through microbial degradation (Tomlin, 2003). Sopeña et
20 al. (2008) found a strong correlation between the soil dehydrogenase activity, and
21 alachlor dissipation in a soil similar to that examined in our study. Felsot and Dzanor
22 (1995) reported that alachlor inhibited this enzyme at very low concentrations, and
23 caused prolonged inhibition for at least 21 d at concentrations $\geq 750 \text{ mg kg}^{-1}$. The
24 amounts of herbicide extracted with methanol account for its bioavailable bound
25 residues, which prolong its herbicidal activity on the microbial flora, because of the low

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

3 The low rate of alachlor dissipation can also be attributed to the generally
4 low temperatures (Fig. 3). Yen et al. (2005) reported that the half-life of alachlor
5 increased from 68 to 187 d when decreasing the incubation temperature decreased from
6 25°C to 10°C at a soil moisture content of 90% of the field capacity. The temperatures
7 recorded during our experiment were in the range of 8- to 13 °C range, with the
8 exception of the last month and a half, which had higher values (~17-20°C).

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

15 This study demonstrates a reduction in the leaching of alachlor from PC-clay
16 and EC formulations compared to that from the commercial formulation. The reduction
17 in leaching was reflected by the larger extracted amounts of herbicide after 191 DAT in
18 the 0- to 10 -cm layer of the soil together with the lower extracted amounts in the 20- to
19 30 -cm depth relative to the commercial formulation. In the top soil layer, bioefficacy of
20 the SRFs was maintained only in the PC-clay formulations.

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

1 may be related to the soil moisture. The occurrence of rainfall is a major factor in
2 herbicide release from granular SRFs immediately after application. The granules must
3 be wetted to enable permeation of the active ingredient through the microcapsule shell
4 and its diffusion into the soil solution before it can move through the soil and be
5 available for pest control. The oscillation in soil moisture content, or the effect of drying
6 and wetting cycles is believed to reduce herbicide release and create several lag times in
7 the release profile, producing sub-threshold concentrations of alachlor in the soil matrix.
8 In addition, EC formulations have an average particle size distribution in the range of
9 100 to 400 μm (Sopeña et al., 2009), which makes the wetting of the granules under low
10 rainfall events more difficult in comparison to PC formulations, which have a particle
11 size of around 2 μm .

12 The model yielded good predictions at times of 99 DAT. For longer times
13 (191 DAT), a discrepancy was recorded between the experimental and predicted
14 amounts of the herbicide in the top soil layer (Table 3). The model overestimated the
15 concentration of alachlor, which may be partly explained by an enhanced degradation of
16 the herbicide following adaptation of the microbial populations. Scorza and Boesten
17 (2005) reduced the half-life of bentazone by 2.5-fold in order to simulate its leaching.
18 Hence, in our study, calculations were performed to account for this effect by
19 decreasing the half-life of alachlor. The inaccuracy in the calculated values was largely
20 reduced by employing a 1.6-fold reduction in its half-life (8 days) for the period
21 between 99 and 191 DAT. The predicted values in the 0- to 10 -cm depth were 21.5%
22 for the commercial formulation, 26.3% for A5/8, 28.3% for A1.6/14, 31.1% for A14
23 and 44.1% for A18. This improved fitting was expressed by a much lower RMSE
24 (7.9% versus 18.0%).

1 Another source of uncertainty in the predictions may arise from herbicide
2 aging in the soil, which the model does not account for: this process does not occur
3 when determining the half-life of alachlor under laboratory conditions due to its
4 relatively rapid degradation. Because of this aging, the fraction of extractable residues
5 decreases at the expense of non-extractable /recalcitrant residues, resulting in a
6 reduction in the bioavailability and degradation of the herbicide (Boivin et al., 2004).
7 This fraction is included in the calculations, but is not susceptible to leaching and
8 degradation processes; therefore, the calculations will yield an overestimation of the
9 residual amounts in the soil, as noted in Table 3. The effect of sequestration of the
10 active compound in the soil is manifested by the difficulty in extracting part of this
11 recalcitrant fraction, which requires increasingly harsh solvents (Taylor et al., 2005).
12 There is a good correlation between bioavailability and the soil fraction extracted with
13 methanol (Kelsey et al., 1997; Barriuso et al., 2004). A sequential extraction after
14 methanol with a mixture of acetone/ethyl acetate/water (AEW) (2:2:1 v:v) will extract
15 some of the recalcitrant fractions (Laabs and Amelung, 2005). This sequential
16 extraction was performed after 191 d for the commercial and the PC-clay formulations,
17 where a longer aging period for the herbicide is expected due to the longer residence
18 time of the free herbicide in the soil. With EC formulations, in which the active
19 ingredient is retained for longer periods within the EC matrix, herbicide aging is less
20 important. The AEW-desorbed fractions in the top soil layer amounted to $15.2\pm 7.4\%$,
21 $10.5\pm 0.3\%$, and $10.1\pm 1.5\%$ for the commercial, A5/8, and A1.6/14 formulations,
22 respectively. These AEW recoveries are indicative of the aging of the herbicide in the
23 soil, but the aged fraction of the herbicide might account for a larger amount than that
24 determined by AEW extraction, since there could be an important fraction of the
25 herbicide incorporated into the soil matrix by abiotic processes.

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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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1 **Figure legends.**

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

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

10 Figure 3. Fortnightly average precipitation (mm rain) (●) and air temperature (°C) (■)
11 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.

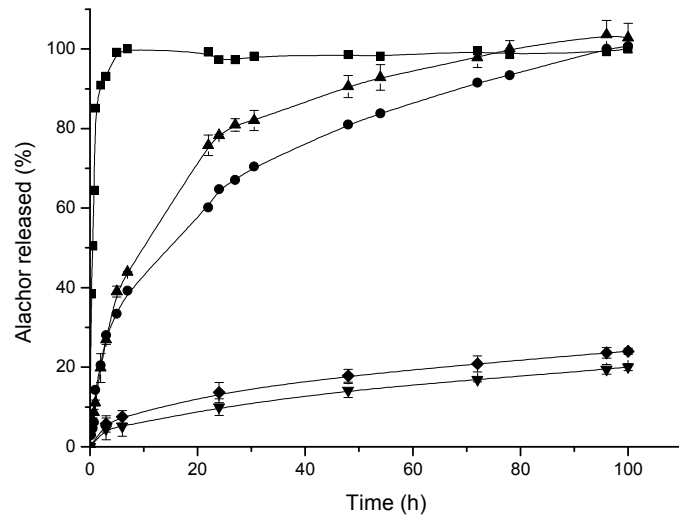


Figure 1

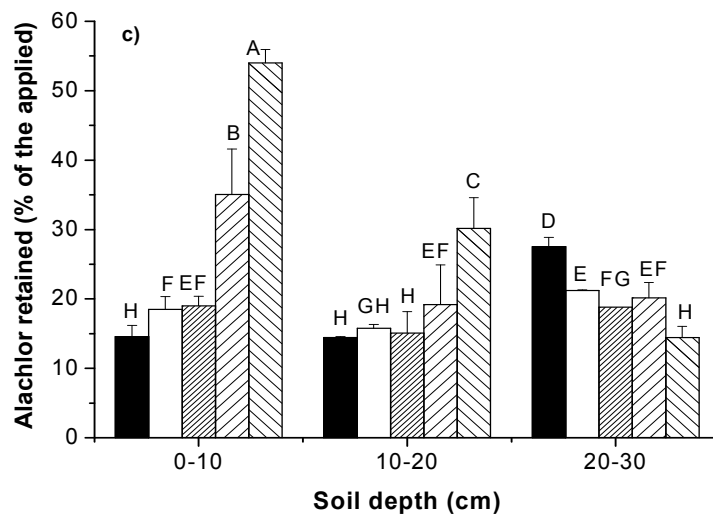
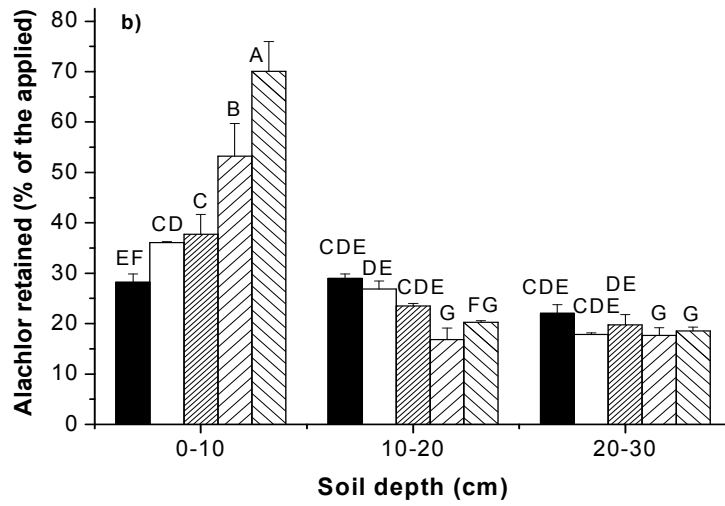
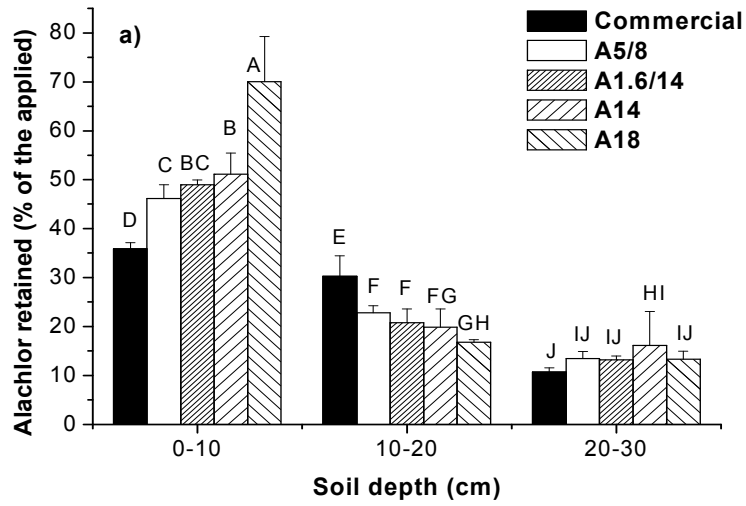


Figure 2

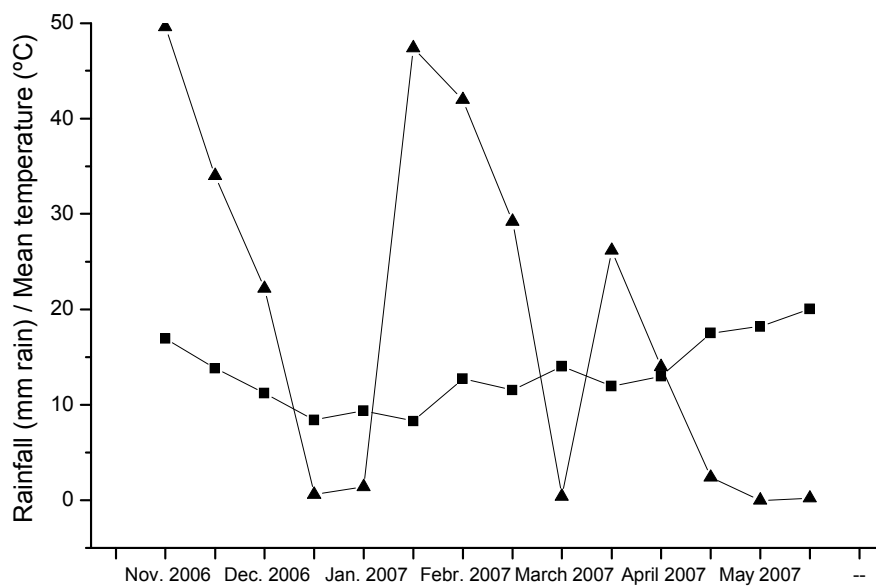


Figure 3