

1 INFLUENCE OF COMMERCIAL FORMULATION ON
2 LEACHING OF FOUR PESTICIDES THROUGH SOIL

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Abstract

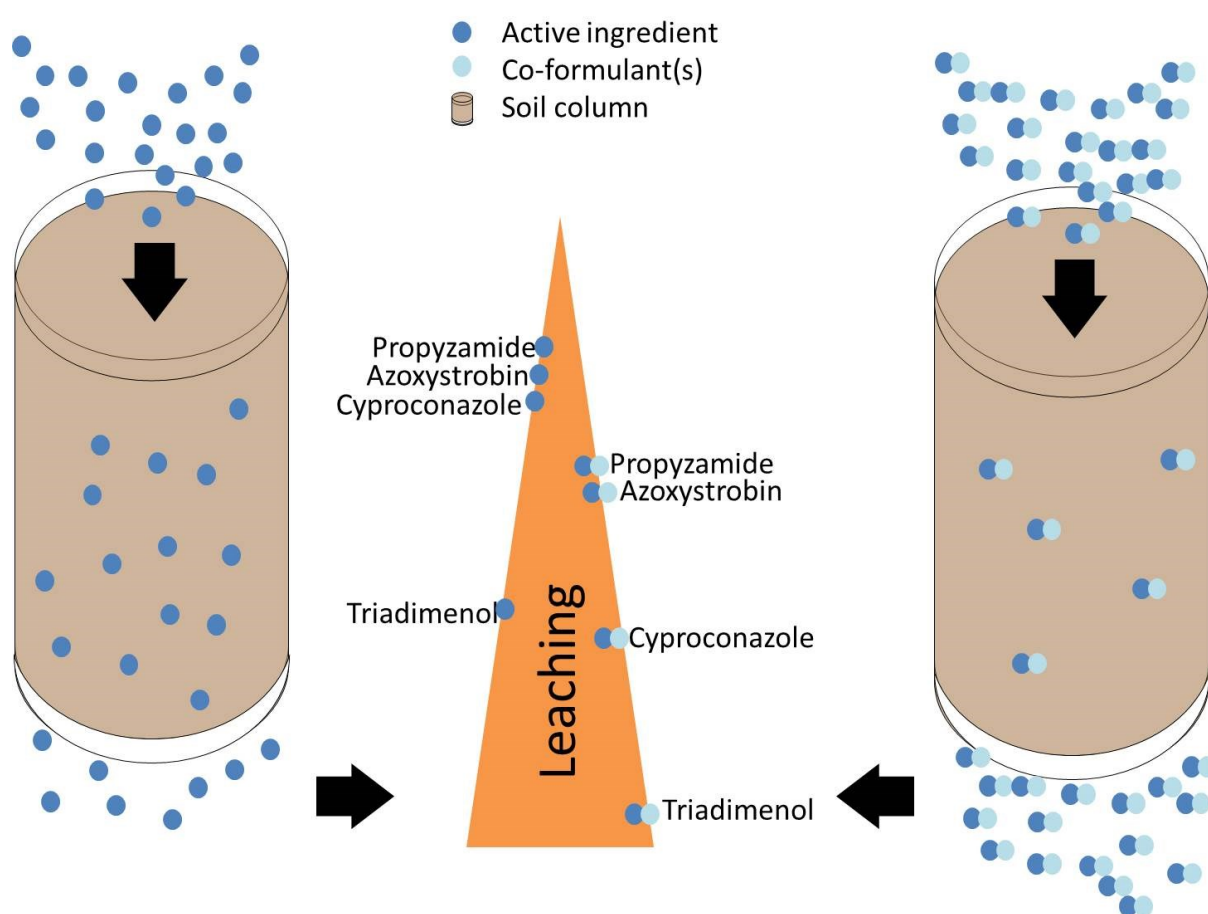
Studies with small soil columns (2 cm i.d. x 5.4 cm depth) compared leaching of four pesticides added either as technical material or as commercial formulations. Pesticides were selected to give a gradient of solubility in water between 7 and 93 mg L⁻¹, comprising azoxystrobin (emulsifiable concentrate, EC, and suspension concentrate, SC), cyproconazole (SC), propyzamide (SC) and triadimenol (EC). Columns of sandy loam soil were leached with 6 pore volumes of 0.01M CaCl₂ either 1 or 7 days after treatment. Separate experiments evaluated leaching of triadimenol to full breakthrough following addition of 18 pore volumes of 0.01M CaCl₂. The mass of pesticide leached from columns treated with commercial formulation was significantly larger than that from columns treated with technical material for all compounds studied and for both leaching intervals (two-sided t-tests, $p < 0.001$). This difference was conserved when triadimenol was leached to full breakthrough with 79 ± 1.2 and $61 \pm 3.1\%$ of applied triadimenol leached from columns treated with formulated and technical material, respectively. There were highly significant effects of formulation for all pesticides (two-way ANOVA, $p < 0.001$), whereas leaching interval was only significant for azoxystrobin EC formulation and cyproconazole ($p < 0.001$ and 0.021, respectively) with greater leaching when irrigation commenced 1 day after treatment. Leaching of azoxystrobin increased in the order technical material (6.0% of applied pesticide) < SC formulation (8.5-9.1% of applied) < EC formulation (15.8-21.0% of applied). The relative difference between leaching of formulated and technical pesticide increased with pesticide solubility in water, increasing from a factor of 1.4 for the SC formulation of azoxystrobin to 4.3 for the SC formulation of triadimenol. Experimental systems differ markedly from field conditions (small columns with intense irrigation). Nevertheless, results indicate the need to consider further the influence of co-formulants in pesticide formulations on behaviour of the active ingredient in soil.

Keywords: Co-formulants, contaminant, environmental fate

Highlights

- Greater pesticide leaching from commercial formulations versus technical material
- Emulsifiable concentrate of azoxystrobin leached more than suspension concentrate
- Effect of formulation on leaching increased with solubility of the active substance
- Assessments of pesticide fate that ignore co-formulants may be over-simplified

Graphical abstract



1. Introduction

Leaching of pesticides through soils may result in contamination of groundwater via transport to depth and surface water via either direct interception by subsurface drains or transfer through the saturated zone as river baseflow. Factors influencing leaching have been investigated over many years and include properties of the pesticide as well as factors relating to soil, hydrogeology, weather and agronomy (Flury, 1996). Given the huge amount of work reported in the literature, it is surprising that the influence of formulating pesticides as commercial products has not received greater attention to date. We previously compared leaching of the herbicide propyzamide through small soil columns when added at the surface either as technical grade pesticide or the commercial product Kerb® Flo which is formulated as a suspension concentrate (Khan & Brown, this issue). We demonstrated enhanced availability in pore water and greater leaching of the commercial formulation in two soils and for intervals between treatment and leaching of 1 to 28 days. Here, we extend the investigation to compare the effect of commercial formulation on leaching of three additional pesticides.

The literature reports a large number of studies that investigate the effect on leaching of incorporating pesticides into controlled-release formulations including manufactured polymers as well as starch, lignin and alginate-based systems (Dubey et al., 2011). This formulation type allows for the slow release of the active substance over time and many experiments compare leaching of controlled-release formulations with that of either technical pesticides or sprayable formulations of pesticides. Generally, it has been shown that controlled-release formulations restrict pesticide leaching by reducing the concentration of the chemical in the bulk soil that is available for leaching (Flury, 1996). There are exceptions in the literature, and controlled-release formulations can extend the period during which pesticide is present in soil and increase leaching when this occurs at longer periods after

application (Buhler et al., 1994). Setting aside this exception of controlled-release formulations, it has often been assumed for other formulation types that the active substance separates from any co-formulants upon entry into the soil and that pesticide fate in soil is unchanged relative to that of the pure active substance (Flury, 1996).

A few recent studies have investigated formulation types other than controlled-release, undertaking batch experiments that compare sorption of pesticides in soil slurry systems when added as pure substances or as commercial formulations (e.g. Beigel & Barriuso, 2000; Pose-Juan et al., 2010; Földényi et al., 2013). We have shown that such experiments will not always capture formulation effects and proposed that a centrifugation methodology provides a better measure of any influence of formulation under natural soil conditions (Khan & Brown, this issue). Where sorption of pesticides has been shown to be less for commercial formulations than for technical material, it has been hypothesised that co-formulants act either to solubilise the active substance or to hold it in solution by formation of relatively stable micelles through the action of surfactants and/or oils (Beigel and Barriuso, 2000; Pose-Juan et al., 2011); in either case, the effect is to retard sorption by holding a greater proportion of the active substance in soil solution. Alternative behaviours have been observed and Pose-Juan *et al.* (2010) reported greater sorption of penconazole from a commercial water-oil emulsion formulation of penconazole compared to technical grade material in batch experiments. They hypothesised that the presence of oil-surfactant mixture in the commercial formulation influenced sorption of penconazole through either enhanced penetration of the active substance onto the less polar sites of the soil organic matter, or by co-adsorption of the active substance within the oil-surfactant mixture.

There are far fewer studies that compare leaching of sprayable formulations with that of the technical material. Wybieralski (1992) carried out column leaching experiments to investigate the kinetics of leaching of propoxur and reported fastest leaching of the

compound from an emulsion form, intermediate leaching kinetics for a suspension form and slowest leaching from pure active substances in five different arable soils. Sharma et al. (2013) compared leaching of hezaconazole under saturated conditions for four soil types; they included a comparison between technical material and a 5% emulsifiable concentrate formulation and reported that leaching results were similar. Any impact of formulation may be particularly significant under conditions of macropore flow, where pesticide can be transferred into infiltrating water close to the soil surface and transported rapidly to depth. Where chromatographic flow through the soil matrix is dominant, the timescales involved may be much longer providing a much greater opportunity for separation of the active substance from any co-formulants.

It is well established that hydrophobic partitioning is an important component of pesticide sorption for low-solubility, non-polar pesticides. This is not an active sorption mechanism, but rather involves a partitioning between soil aqueous phase and a non-specific surface of a solute introduced into the soil environment (Gevao *et al.*, 2000; Kah and Brown, 2006). Hydrophobic bonding may also be regarded as solvent-motivated sorption where a hydrophobic solute is expelled from the water and becomes associated with the soil organic matrix. By changing the microenvironment surrounding a hydrophobic pesticide in soil solution, co-formulants could be expected to affect a change on sorption to soil constituents, and it might further be expected that the extent of this effect would be different for chemicals with different solubility in water. Here, we investigate the effect of commercial formulation on leaching of three pesticides through small soil leaching columns and compare results with those generated previously for propyzamide. Experiments aim to determine whether the observation of enhanced leaching of propyzamide from a commercial formulation compared to the technical grade pesticide can be generalised and whether any effect varies as a function of pesticide solubility in water.

2. Materials and methods

2.1. Test materials

A sandy loam soil (62% sand, 37% silt, 1% clay) of the Blackwood association was collected from the upper 5-15 cm of an arable field margin in York, UK (national grid reference 4648 4478). The soil has pH in water of 5.1, organic matter content of 2.7%, and water contents at 100 cm water tension and maximum water holding capacity of 0.22 and 0.37 g g⁻¹, respectively. Soil was air dried at room temperature, passed through a 2-mm mesh sieve, and stored at <5°C in the dark prior to use.

Pesticides used in this study were chosen to provide a gradient from low to moderate solubility in water whilst maintaining similar sorption characteristics and being stable to aqueous photolysis and relatively persistent in soil. Physico-chemical properties of the pesticides studied are summarised in Table 2. Analytical grade propyzamide, azoxystrobin and triadimenol (purity 99.6, 99.9 and 98.7%, respectively) were purchased from Sigma-Aldrich Ltd (Dorset, UK), while cyproconazole (racemic mixture of diastereomers A and B; purity 99.7%) was acquired from Alfa Aesar (UK). The commercial products Kerb® Flo (suspension concentrate (SC), 400 g L⁻¹ (35.3% w/w) propyzamide), Priori Xtra® (SC mixture of 200 g L⁻¹ azoxystrobin and 80 g L⁻¹ cyproconazole), Headway™ (emulsifiable concentrate (EC) mixture of 62.5 g L⁻¹ azoxystrobin and 104 g L⁻¹ propiconazole) and Veto® F (EC mixture of 75 g L⁻¹ triadimenol and 225 g L⁻¹ tebuconazole) were supplied by the Food and Environment Research Agency York, UK. Information on the co-formulants declared on the product labels are given in Table 2.

Deionised water was used to make up CaCl₂ solution for leaching experiments. All solvents used throughout and water used in pesticide extraction procedures were HPLC grade.

2.2. Leaching experiments

Leaching experiments were carried out in PVC columns (2 cm i.d. x 10 cm length). Each column was manually packed with 20 g of soil on an oven-dry weight basis with glass wool at the bottom to prevent loss of soil. Four replicate columns were prepared per treatment and for each leaching event. Soil was added to the columns in steps followed by gentle tapping to ensure uniform packing and achieve the same height of soil within each column (ca. 5.4 cm). Columns were then saturated from the base with 0.01M CaCl_2 and allowed to drain freely for 24 h under gravity. After this time, water held within the glass wool plug was evacuated by applying a small suction to the base of the column using a plastic syringe. The bulk density of the packed column was 1.18 g cm^{-3} and this value was used to determine total porosity of $0.54 \text{ cm}^3 \text{ cm}^{-3}$ based on an assumed particle density for topsoil of 2.55 g cm^{-3} (Avery and Bascomb, 1982). One pore volume for each soil column was thus equivalent to 9.20 cm^3 .

Eight soil columns each were treated with either technical or formulated material for azoxystrobin (EC and SC formulations), cyproconazole, propyzamide, and triadimenol, giving a total of 72 treated columns. A further four columns were untreated to act as controls. Each treated column received $100 \text{ }\mu\text{g}$ of the respective pesticide. This is equivalent to $3.2 \text{ kg a.s. ha}^{-1}$ which is larger than typical field application rates and was selected to facilitate analysis for pesticides in leachate. Columns receiving technical pesticide were treated with 0.1 mL of a $1000 \text{ }\mu\text{g mL}^{-1}$ solution in acetone; the solvent was allowed to evaporate before the addition of 0.5 mL deionised water. Columns receiving formulated pesticide were treated with 0.5 mL of a $200 \text{ }\mu\text{g active substance mL}^{-1}$ solution of the formulated material in deionised water. Immediately after treatment, a 1-cm layer of acid-washed sand was added to the top of each column. All columns were incubated in the dark and at $<5^\circ\text{C}$ until leaching.

Separate columns were leached either 1 or 7 days after pesticide application. At each leaching event, one set of four replicates from each treatment together with two control columns were irrigated with a total of six pore volumes (55.2 mL) of 0.01M CaCl₂ solution using a 323S Watson Marlow peristaltic pump at the rate of 12.6 mL hr⁻¹. Rate of irrigation was equivalent to 40.1 mm hr⁻¹ which is at the upper end of natural rainfall intensities in temperate maritime climates such as that in the UK. Irrigation did not result in a continuous layer of water on the soil surface. Leachate was collected as a single fraction at the bottom of each column, weighed and stored in the fridge pending analysis.

A separate column leaching experiment was carried out with triadimenol to generate complete breakthrough curves for this compound. The aim was to determine whether the effect of pesticide formulation was to induce only a change in the timing of breakthrough or whether the total loss of pesticide in leachate was also changed. Eight soil columns were prepared and treated as described above, with four each treated with either technical or formulated triadimenol. All columns were leached 24 hours after pesticide application with a total volume of 165.6 mL of 0.01M CaCl₂ solution, equivalent to 18 pore volumes (323S Watson Marlow peristaltic pump at a rate of 12.6 mL hr⁻¹). Leachate was collected in 10-mL fractions for separate analysis.

2.3. Sample preparation for analysis

For analysis of propyzamide in leachate, a 1-mL aliquot of each leachate sample was transferred into a test tube and evaporated to dryness under a gentle stream of nitrogen on a sample concentrator at room temperature. The residue was re-dissolved in 1 mL of hexane prior to analysis by GC-MS.

Leachate from columns treated with azoxystrobin was concentrated using solid-phase extraction (SPE), based on a method adapted from Montagner et al. (2014). Oasis HLB

cartridges (6 cm³) packed with 200 mg of 30 µm sorbent material were conditioned with 5 mL each of methanol followed by acetonitrile followed by water using a 12-port SPE vacuum manifold (Supelco, UK). Leachate samples were passed through cartridges at 1 mL min⁻¹. After sample loading, cartridges were dried under vacuum for 20 mins and eluted with 4 mL methanol followed by 4 mL acetonitrile. Samples were evaporated to dryness under nitrogen and re-dissolved into 2 mL ethyl acetate. A further fourfold dilution step in ethyl acetate was undertaken prior to analysis by GC-MS.

Leachate samples containing triadimenol and cyproconazole were also concentrated using SPE. Oasis HLB cartridges (6 cm³) were pre-conditioned with 2 x 5 mL acetonitrile followed by 2 x 5 mL water. After loading samples, cartridges were dried under vacuum for 20 mins and pesticides eluted with 5 mL acetonitrile. Samples were evaporated to dryness under nitrogen and then re-dissolved into 1 mL methanol. Subsequent dilutions in methanol (eight- to 28-fold) were required to deliver all samples for analysis within the range of the calibration standards.

2.4. Pesticide analysis

Propyzamide and azoxystrobin were analysed by GC-MS. A PerkinElmer (Cambridge, UK) Clarus 680 gas chromatograph equipped with a Clarus 600 mass spectrometer and automatic split-splitless injector was operated in electron impact ionization mode with an ionizing energy of 70eV, scanning from m/z 50-500 at 0.2 s per scan. The ion source temperature was 180°C. The electron multiplier voltage was maintained at 323 V, and a solvent delay of 3 min was employed. An Elite-5MS fused silica capillary column (30 m x 0.25 µm i.d. and 0.25 µm film thickness) supplied by PerkinElmer was used. The oven temperature was maintained at 90°C for 1 min, followed by a ramp to 270°C at a rate of 20°C min⁻¹ for propyzamide, while for azoxystrobin, the oven temperature was programmed as

45°C for 1 minute, followed by a ramp to 295°C at 25°C min⁻¹ and held for 5 minutes. Helium was used as carrier gas with a flow rate of 20 mL min⁻¹ for both chemicals. Sample (1 µL) was injected in splitless mode and the injector temperature was maintained at 250°C. There were six pre- and post-injection needle washes were programmed at six respectively. Analysis was performed with selected ion monitoring (SIM) mode using primary mass ions of m/z 173 and 344 for quantification of propyzamide and azoxystrobin, respectively. The target and qualifier abundances were determined by injection of propyzamide and azoxystrobin standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from m/z 50-500. Compounds were confirmed by their retention times and the identification of target and qualifier ion. Retention times had to be within 0.2 min of the expected time, and qualifier-to-target ratios had to be within a 10% range for positive confirmation. The limit of detection (LoD) and limit of quantification (LoQ) for propyzamide were 0.02 and 0.05 µg mL⁻¹, respectively whilst those for azoxystrobin were 0.1 and 0.4 µg mL⁻¹, respectively.

Triadimenol and cyproconazole were analysed on a PerkinElmer Flexar Chromera HPLC system with UV-vis detector and a Supelco 516 C-18-DB column (15 cm x 4.6 mm x 5 µm). The mobile phase was an isocratic mixture of methanol and water (70:30 v/v) at a flow rate of 1.0 mL min⁻¹. The injection volume was 20 µL and the oven temperature was maintained at 30°C. All analytes were determined at 221 nm. The retention time for triadimenol was 5.4 minutes whilst those for diastereomers A & B of cyproconazole were 4.7 and 5.3 min, respectively. The LoD and LoQ for triadimenol were 0.3 and 0.7 µg mL⁻¹, respectively, whilst those for cyproconazole were 0.4 and 0.9 µg mL⁻¹, respectively.

2.5. Data analysis

Leaching behaviour was assessed based on the total mass of pesticide lost in leachate from individual soil columns. In addition, the relative difference in leaching from columns treated with formulated and technical pesticide was calculated by dividing the mean mass of pesticide in leachate from the former by that from the latter. Datasets were tested for normality using the Shapiro-Wilk test. Two-sided t-tests were used to investigate differences in losses from columns treated with technical grade and commercial formulations of individual pesticides. Two-way and multi-way ANOVA were carried out to test the significance of formulation and interval between application and irrigation on the leaching behaviour of pesticides. Tukey post-hoc tests were undertaken to test for differences between individual treatments over time; this is a pairwise comparison, similar to a t-test, but designed to compensate for the increased likelihood of a significant result occurring due to chance as the number of comparisons increases. All statistical analyses were undertaken in SPSS (IBM Analytics).

3. Results

3.1 Influence of formulation on leaching behaviour

The total volume of leachate collected from individual columns ranged between 53.28 and 55.76 mL (96.5 and 101.0% of irrigation volume). There were no significant differences between treatments or between leaching time intervals (2-sided t-test).

Fig. 1 presents mass recovery for pesticides in leachate. Diastereomer A and B of cyproconazole were quantified separately, but are combined within Fig. 1 for simplicity. Statistical analyses reported below were undertaken both for each individual diastereomer and for the combination of the two. Leaching of the technical material was similar for azoxystrobin, cyproconazole and propyzamide (e.g. 6.0-7.8% of applied for irrigation 1 day

after treatment) despite a range in representative Koc of 364-840 mL g⁻¹. Triadimenol leached to a much greater extent despite having a representative Koc value of 750 mL g⁻¹ (Table 1); this anomaly in behaviour was not investigated further. For reference, the average penetration depth (e.g. Leonard & Knisel, 1987) through the columns under the experimental conditions can be calculated at 2.2 cm assuming piston flow for a pesticide with Koc of 500 mL g⁻¹. This value is consistent with the relatively small masses leached from the technical grade treatments (with the notable exception of triadimenol).

The mass of pesticide lost from treatments with commercial formulations of pesticide was consistently larger than that from treatments with technical pesticide (Fig. 1). This result applied for all compounds studied and for both leaching intervals and was highly statistically significant in every instance (two-sided t-tests, p<0.001). Two different formulations were investigated and compared with the technical treatment for azoxystrobin. Mass of pesticide leached from the three treatments (Fig. 1) were statistically different at both leaching intervals (two-sided t-tests) and increased in the order technical pesticide (6.0% of applied pesticide) < Priori Xtra® soluble concentrate (SC) formulation (8.5-9.1% of applied pesticide) < Headway™ emulsifiable concentrate (EC) formulation (15.8-21.0% of applied pesticide).

Absolute differences in pesticide leaching after 1 and 7 days were relatively small within any single treatment (maximum decrease in leached loss at 7 days relative to 1 day was 21% for propyzamide formulated material). Two-way ANOVA was used to test the effects of formulation and interval between application and irrigation on the leaching of individual pesticides (Table 3). The effect of formulation was highly statistically significant with p-values of <0.001 for all pesticides. The effect of interval to irrigation was statistically significant for azoxystrobin EC formulation and cyproconazole (p-values <0.001 and 0.002, respectively), but not azoxystrobin SC formulation, propyzamide or triadimenol. Interaction

between formulation and interval to irrigation was statistically significant for azoxystrobin EC formulation and cyproconazole (p-values <0.001 and 0.021, respectively), but not for the other chemicals. A multi-way ANOVA across the whole dataset showed highly statistically significant effects of pesticide type, formulation and an interaction between these two factors (all $p < 0.001$). There was no effect of interval to irrigation on leaching ($p = 0.096$), and no interaction between formulation and interval to irrigation on leaching ($p = 0.584$). However, the interaction of formulation and pesticides was highly statistically significant ($p < 0.001$).

3.2 Influence of formulation as a function of pesticide solubility

The ratio of the mass leached from the formulated treatment to that from the technical treatment was calculated (section 2.5) and is plotted in Fig. 2. All values were larger than one because leaching was greater from commercial formulations than from their equivalent treatments with technical pesticides in all cases. Values for the ratio of mass leached from formulated and technical treatments ranged between 1.4 for the SC formulation of azoxystrobin and 4.3 for the SC formulation of triadimenol. For our set of chemicals (solubility range $6.7 - 93 \text{ mg L}^{-1}$), there was a clear trend of larger relative differences in leaching for more soluble compounds. In contrast, there was no consistent pattern in the relative effect of formulation on leaching for the two leaching intervals. Sometimes the ratio of mass leached from formulated and technical treatments was greater at day 1 and sometimes at day 7.

3.3 Leaching of triadimenol through to full breakthrough

Separate soil columns treated with triadimenol were leached with 18 pore volumes of 0.01M CaCl_2 to investigate whether the effect of formulation on leaching was solely to retard leaching or also to influence the total amount of pesticide leached through soil. Breakthrough

curves (Fig. 3) showed more rapid leaching for columns treated with formulated material than for those treated with technical pesticide; breakthrough occurred in the 20-30 mL fraction for formulated material and in the 30-40 mL fraction for technical pesticide, whilst maximum leached mass was in the 30-40 and 40-50 mL fractions for formulated and technical materials, respectively. There was also a large difference in the mass of triadimenol leached across the experiment. Total leached loss from columns treated with formulated material was $79 \pm 1.2\%$ of applied, whilst that from columns treated with technical material was $61 \pm 3.1\%$ of applied. The maximum concentration of triadimenol in any fraction of leachate was 2.6 ± 0.13 and $1.2 \pm 0.13 \mu\text{g mL}^{-1}$ for columns treated with formulated and technical materials, respectively. Total leaching of triadimenol from both treatments in the full breakthrough experiment (ca. 18 pore volumes; Fig. 3) was less than that from the main experiment (6 pore volumes; Fig 1). This suggests some variability in the hydraulic properties of the soil columns in the two experiments, but does not detract from the main findings of the study which always rely on comparison between treatments within a single experiment.

4. Discussion

The most important finding from this research is that we observed enhanced leaching of all four pesticides from their commercial formulations relative to the technical materials. Relative differences in leached masses ranged between factors of 1.4 and 4.3, and all differences were highly statistically significant and were observed for leaching both 1 and 7 days after treatment (Fig. 1 and Table 3). Examples of similar studies in the literature are rare. Our results are in accordance with those of Wybieralski (1992) who reported faster washout from soil columns by leaching of propoxur for emulsifiable and suspension formulations than for the technical material. The extended leaching experiment reported here for triadimenol adds to this early study by demonstrating that not only was breakthrough of

the pesticide observed earlier for the formulated treatment, but also both peak concentration and total mass of pesticide lost via leaching were larger for the commercial formulation than for the technical material. Co-formulants in the emulsifiable concentrate product of triadimenol influenced the initial availability of the active substance for leaching, resulting in earlier breakthrough; centrifugation experiments have shown a similar effect of a suspension concentrate formulation on availability of propyzamide in pore water (Khan and Brown, this issue). The full breakthrough experiment with triadimenol also indicates that the effect of co-formulants extends beyond influencing initial availability; less of the active substance became available for leaching for the technical treatment over the course of column elution with 18 pore volumes, implying a substantive change to sorption for at least a part of the active substance applied to soil.

Rank order for leaching losses of azoxystrobin reported here (Fig. 1) match the observations of Wybieralski (1992), with leaching increasing in the order technical material < suspension concentrate (Priori Xtra®) < emulsifiable concentrate (Headway™). Emulsifiable concentrate (EC) formulations comprise a solution of pesticide with emulsifying agents in a water insoluble organic solvent which is designed to form an oil-in-water emulsion upon dilution (Mulqueen, 2003; Knowles, 2008). The presence of oily water-insoluble organic solvents in EC formulations may affect the behaviour of pesticide active substances in two ways. First, it may restrict the pesticide molecule from dissolving in water and secondly, the oily organic solvents surrounding the pesticide molecule may retard processes controlling sorption to soil. Suspension concentrate (SC) formulations, also called flowables, usually comprise particles covering a wide size range (0.1-10 µm) dispersed in a liquid medium, generally water, at high solid volume fractions (up to 60%) and usually contain suspension agents, wetting agents and thickeners (Luckham, 1989).

Environmental fate of pesticides in soil is known to be influenced by solubility of the active substance in water (Delle Site, 2001; Fernández-Pérez et al., 2011; Langeron et al., 2014). Hydrophobicity of non-ionic pesticides can be a significant driver for partitioning of pesticides out of soil solution (Kah and Brown, 2006). It was plausible to hypothesise that any effect of co-formulants that increased the availability of the active substance in soil solution would be greater for pesticides with smaller solubility in water. In fact, the experiments demonstrated the opposite effect, with the difference in leaching between formulated and technical treatments being largest for pesticides with larger solubility (Fig. 2). We selected pesticides with low to moderate solubility (6.7 to 93 mg L⁻¹) on the basis that solubility could be a limiting factor for availability in soil pore water soon after application. The mechanisms responsible for our findings are unclear, though it should be noted that we did not control for formulation ingredients so there may have been some systematic change in either the nature or concentration of co-formulants for the four active substances along our gradient of solubility.

As noted by Khan and Brown (this issue), the leaching experiments controlled for most aspects of study design but not for the means of initial addition of test materials to soil. All commercial formulations were added to columns in 0.5 mL 0.01M CaCl₂ solution, whereas technical materials were added in 0.1 mL acetone which was allowed to evaporate prior to adding pesticide-free CaCl₂ solution to match that added in the formulated treatments. Dosing in acetone is a possible source for experimental artefacts if, for example, the solvent resulted in enhanced transfer of pesticide into soil aggregates or precipitation of propyzamide on soil surfaces. A further possible confounder in the current experiments is that several of the commercial formulations contained a second active substance (Table 2). If there was competition for sorption then this could have exacerbated leaching from commercial formulations. This is considered plausible but unlikely on the basis of the

literature to date; there are occasional studies that investigate competition for sorption, but results are conflicting. Turin and Bowman (1997) investigated competition effects between herbicides by saturating other herbicides to 50% of their maximum sorbed amount; under these highly unrealistic conditions, they reported weaker sorption of bromacil and napropamide but not prometryn in the presence of the other herbicides. An opposite behaviour was shown by Tan and Singh (1995), with weaker leaching of norflurazon and either no effect or weaker leaching of bromacil when applied in combination with diuron, metolachlor, oxadiazon, simazine, terbacil or trifluralin. Ebato and Yonebayashi (2005) developed a method specifically to investigate competition effects, but reported that there was no competition for sorption between atrazine and linuron. Thus, evidence for direct competition for sorption between active substances is sparse; any such competition between active substances in a single product could itself be considered a consequence of formulation into a commercial product that is not assessed routinely within risk assessment procedures. The strongest evidence that results were not due to experimental artefacts comes from the comparison of two formulations and technical material for azoxystrobin. Not only were there differences between formulated and technical material, but there were also large and consistent differences in behaviour of the two formulation types, confirming a genuine impact of co-formulants on leaching of the active substance.

5. Conclusion

Experiments have demonstrated a highly significant and consistent effect of commercial formulation on the leaching behaviour of four pesticides relative to the pure technical active substances in systems comprising small soil columns with intense irrigation applied 1 or 7 days after pesticide treatment. Relative differences in leaching between formulated products and technical materials ranged between factors of 1.4 and 4.3, and

differences were larger for active substances with greater solubility in water. Leaching to full breakthrough with triadimenol indicated that co-formulants influence both the initial and the total availability of pesticide for leaching; thus breakthrough from the formulated treatment occurred earlier and leached a greater total mass of the compound relative to the technical treatment. Experiments with two contrasting formulations of azoxystrobin showed differentiated influence on leaching for emulsifiable concentrate and suspension concentrate formulations. Results are reported for small, controlled systems with intense irrigation and these need to be scaled up to investigate behaviour under conditions closer to the field. Potentially, there are important implications for the assessment of environmental fate of pesticides which has been based to date on the assumption that sprayable formulations do not influence behaviour of the active substance in soil.

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Table 1

Key physicochemical properties of the pesticides studied (Source: University of Hertfordshire, 2016)

Properties	Azoxystrobin	Propyzamide	Triadimenol	Cyproconazole
CAS number and name	[131860-33-8] methyl (α E)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]- α -(methoxymethylene) benzeneacetate	[23950-58-5] 3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide	[55219-65-3] β -(4-chlorophenoxy)- α -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	[94361-06-5] α -(4-chlorophenyl)- α -(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
Pesticide type and substance group	Fungicide, strobilurin	Herbicide, benzamide	Fungicide, triazole	Fungicide, triazole
Molecular mass (g mol ⁻¹)	403.4	256.1	295.8	291.8
Solubility in water (mg L ⁻¹) at 20°C	6.7	9.0	72.0	93.0
Log octanol-water partition coefficient (Log P at pH 7, 20°C)	2.5	3.3	3.2	3.1
Soil organic carbon sorption coefficient (K _{oc} , mL g ⁻¹)	589	840	750	364
Degradation half-life in aerobic soil at 20°C (days)	85 [persistent]	47 [moderately persistent]	137 [persistent]	142 [persistent]
Stability to aqueous hydrolysis	Stable	Stable	Stable	Stable
Half-life for photolysis in water (days)	9 [moderately fast]	41 [stable]	9 [moderately fast]	40[stable]

Table 2

Information on formulation types and declared co-formulants for the commercial products used in experiments

Active substance	Formulation	Additional active substance	Declared co-formulants	CAS of co-formulant	Principal role of co-formulant
Azoxystrobin	Headway™ Emulsifiable concentrate	Propiconazole (104 g L ⁻¹)	Tetrahydrofurfuryl alcohol (0.5 ppm)	97-99-4	Solvent
Azoxystrobin (200 g L ⁻¹)	Priori Xtra® Suspension concentrate	Cyproconazole (80 g L ⁻¹)	C16-18 alcohols, ethoxylated (10-20% w/w) Propane-1,2-diol (1-6% w/w) Naphthalene sulfonic acid,, dimethyl-polymer with formaldehyde and methyl- naphthalenesulfonic acid, sodium salt (1-5% w/w)	68439-49-6 57-55-6 9008-63-3	Nonionic surfactant Solvent Dispersant
Cyproconazole (80 g L ⁻¹)	Priori Xtra® Suspension concentrate	Azoxystrobin (200 g L ⁻¹)	See above	See above	See above
Propyzamide (400 g L ⁻¹)	Kerb® Flo Suspension concentrate	None	Propylene glycol (<5%)	57-55-6	Solvent
Triadimenol (75 g L ⁻¹)	Veto® F Emulsifiable concentrate	Tebuconazole (225 g L ⁻¹)	2-ethylhexanol propylene ethyleneglycol ether (1-25%) N,N-dimethylcapramide and N,N-dimethylcaprylamide (>1 - <25%)	64366-70-7 14433-76-2 & 1118-92-9	Nonionic surfactant Solvent

Table 3

P-values from two-way ANOVA for the effects of formulation, interval between application and irrigation and interaction between formulation and interval on the leaching behaviour for the studied pesticides.

Pesticides	Formulation	Interval to irrigation	Formulation x Interval to irrigation
Azoxystrobin SC	<0.001	0.400	0.438
Azoxystrobin EC	<0.001	<0.001	<0.001
Propyzamide	0.001	0.221	0.526
Triadimenol	<0.001	0.515	0.771
Cyproconazole	<0.001	0.002	0.021
- Diastereomer A	<0.001	0.001	0.028
- Diastereomer B	<0.001	0.018	0.031

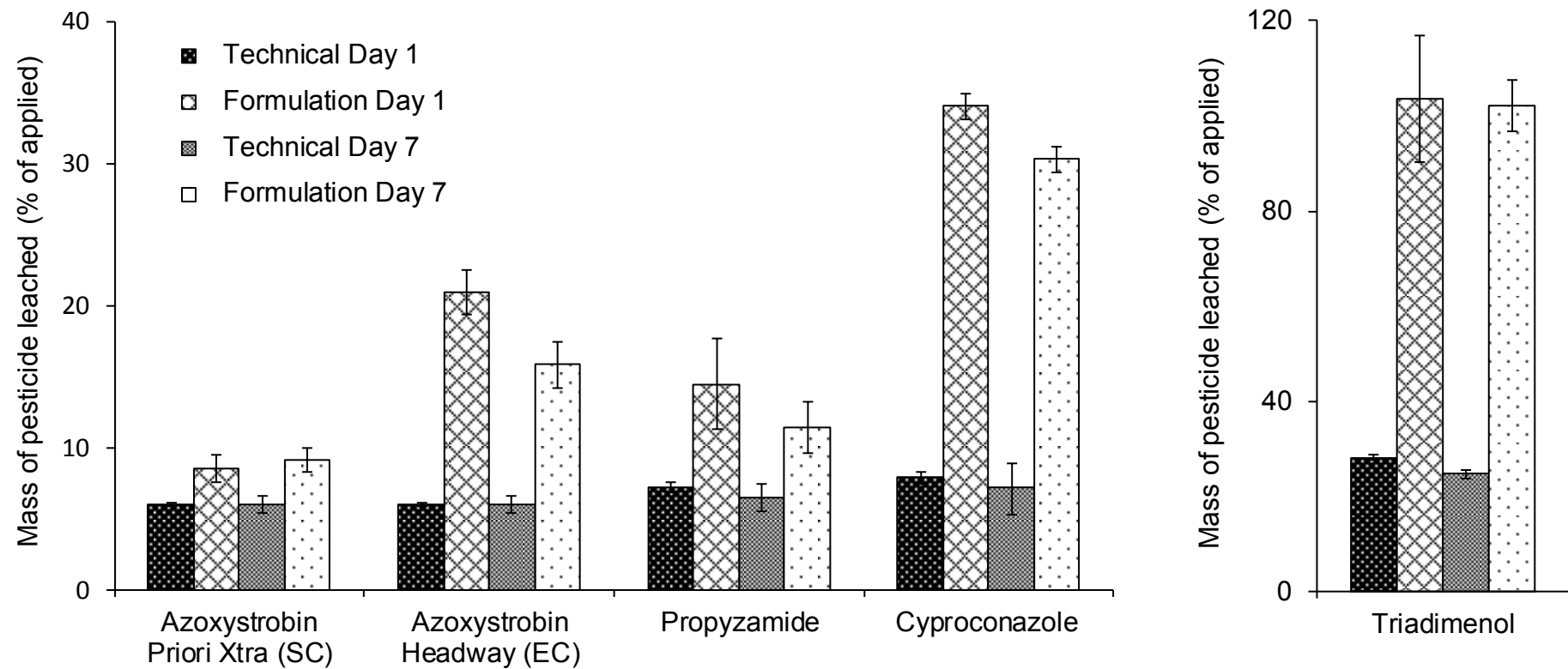


Fig. 1. Mass of pesticides leached through sandy loam soil treated with technical grade and commercial formulations of different pesticides. Error bars are ± 1 standard deviation for four replicates.

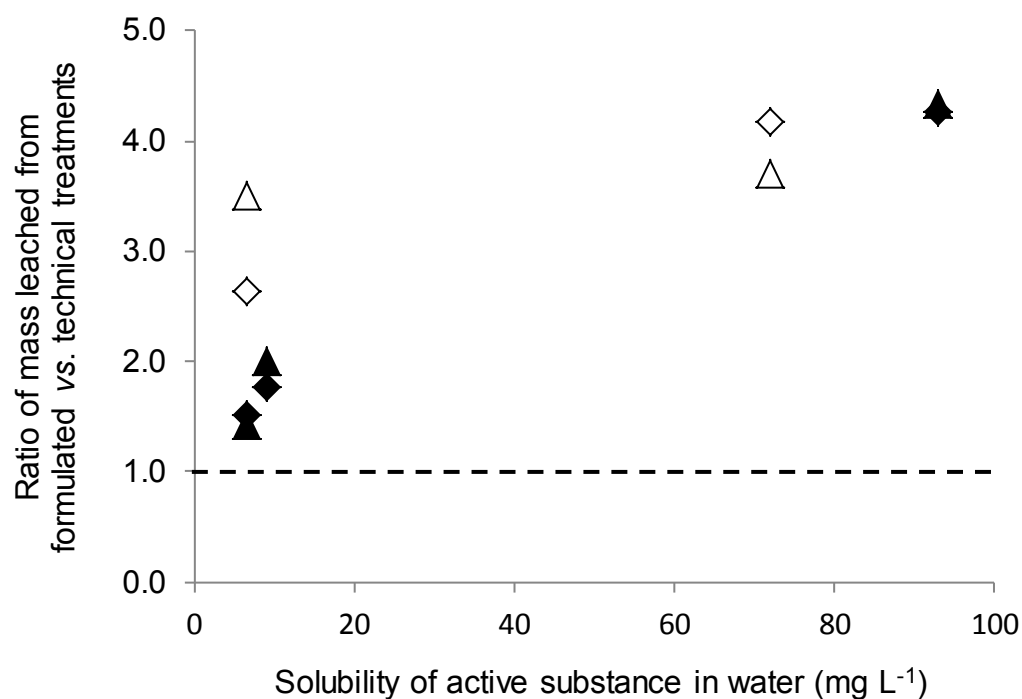


Fig. 2. Comparison of the relative difference in mass of pesticide leached between commercial formulations and technical material grade as a function of solubility in water of the active substance. Closed and open symbols are for suspension concentrate and emulsifiable concentrate formulations, respectively. Triangles and diamonds are for leaching 1 and 7 days after treatment, respectively. All values are above 1.0, indicating greater leaching from the commercial formulation than from the technical material.

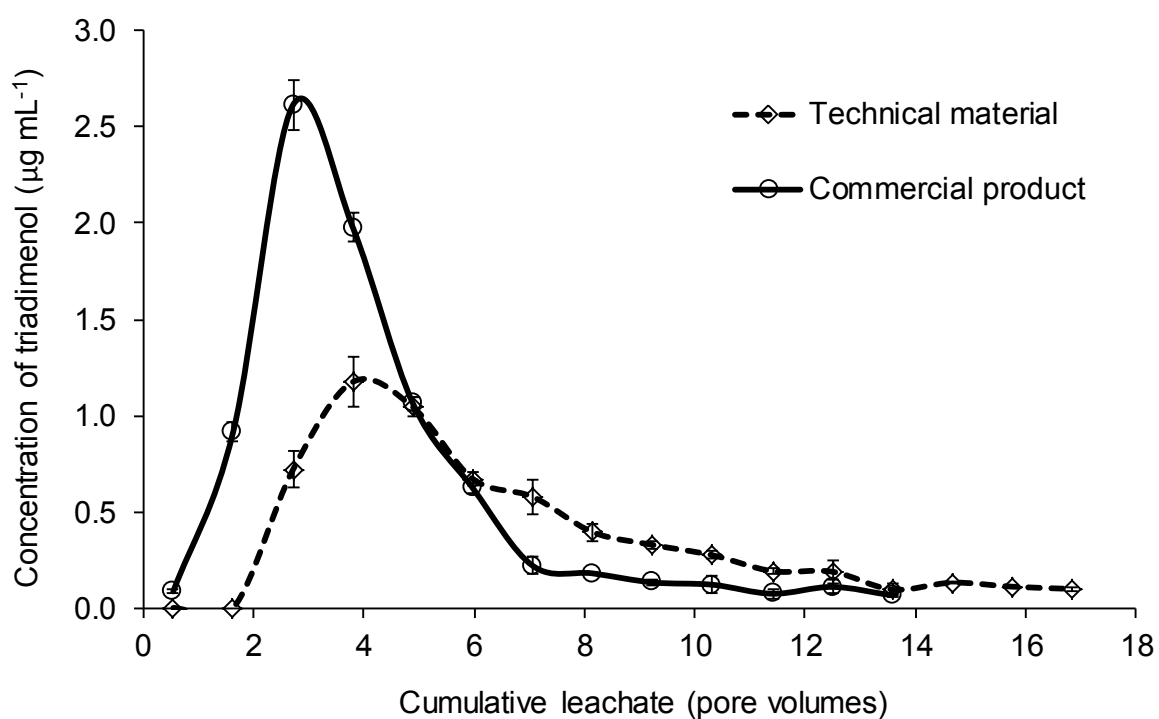


Fig.3. Breakthrough curves of triadimenol following application of technical grade or commercial formulation to soil columns. Error bars are ± 1 standard deviation of four replicates.