Sorption of the nematicide fluensulfone in six UK arable soils – implications for control of the potato cyst nematode *Globodera pallida*

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Running title: Sorption of nematicide fluensulfone in UK arable soils

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Summary - Batch adsorption experiments were performed to determine the sorption of the nematicide fluensulfone in the technical-grade form and granular formulation (as Nimitz 15G®) in six UK arable soils. The Freundlich and equilibrium sorption coefficients K_F and K_D, respectively, were generally low. K_F and K_D correlated positively with soil organic matter in all instances. The sorption kinetics were similar for both forms, but the K_D was about four times lower for Nimitz 15G than the technical-grade, suggesting concentration dependency of fluensulfone sorption. The low sorption of fluensulfone across the soils, indicates that partitioning of fluensulfone to the soil liquid phase may be unlimited. Therefore, substantial availability in the soil to be effective is likely. Sorption, therefore, may not limit fluensulfone efficacy. Nonetheless, these results call for cautious use of the nematicide because leaching is possible.

Keywords - Adsorption; efficacy; *Globodera* species; Nimitz

Sorption is a key factor which influences a pesticide's environmental fate and biological activities (Kravvariti *et al.*, 2010; Wang & Keller, 2009; Villaverde *et al.*, 2008). Sorption may involve both adsorption processes representing uptake of molecules onto the outer surfaces of soil particles (Calvet, 1989) and absorption processes where a pesticide permeates the soil matrix (Thompson. & Gone, 2012). Irrespective of the form in which it manifests, sorption entails the removal of a pesticide from the soil solution (Sposito, 2008), and could constitute a limiting factor to nematicide efficacy (Smelt & Leistra, 1992) since nematodes are essentially aquatic organisms that exist in the soil water.

Fluensulfone is the active ingredient in Nimitz® a recent non-fumigant and contact nematicide from ADAMA Agricultural Solutions Ltd, and is currently registered for use as a pre-planting treatment to solanaceous and cucurbitaceous crops in USA, Canada, Mexico, Dominican Rep., Panama, Israel, Japan, Taiwan, S. Korea, and Australia Danny Karmon, Senior Innovative Product Manager, ADAMA Agricultural Solutions Ltd. Israel. *Personal Communication).* Fluensulfone is favoured to the organophosphate fosthiazate and the carbamate oxamyl nematicides, for its lesser acute toxicity for oral ingestion, dermal contact and inhalation, and is deemed to have low toxicological activity on birds, fish, and bees (Danny Karmon, Senior Innovative Product Manager, ADAMA Agricultural Solutions Ltd. Israel. *Personal Communication*). The activities of fluensulfone have been reported on root-knot nematode, *Meloidogyne* species (Oka *et al*., 2009, 2012, 2013; Morris *et al*., 2015), migratory nematodes, *Pratylenchus penetrans*, *P. thornei* and *Xiphinema index* (Oka, 2014), and the potato cyst nematode *Globodera pallida* (Norshie *et al*., 2016). The mode of action is reported to involve cessation of feeding, paralysis and complete death (Kearn *et al*., 2017).

Fluensulfone is a fluorinated molecule, and the influence of fluorine on a molecule's polarity is known to involve improvement of a molecule's lipophilicity (Dinoiu, 2006; Ichino *et al*., 1990), and allows changes towards non-polarity (Smart, 2001). With fluensulfone deemed to be lipophilic, it is presumed that its sorption to a soil shall depend mainly on the soil's organic matter content. A direct relationship between soil organic peat amendments and fluensulfone efficacy for control of *Meloidogyne* spp. on tomato has been reported by Oka *et al*., (2013). Yet, fluensulfone sorption in soil remains to be measured. The experiments reported in this paper were part of a broad study which evaluated the efficacy of fluensulfone in the management *G. pallida*. The objectives were to (i) determine the sorption of fluensulfone as a function of soil type (ii) compare the sorption of the technical-grade form to that of the granular formulation (as Nimitz 15G®), and (iii) determine the sorption of fluensulfone in soil following organic treatment.

Materials and methods

SUBSTRATES STUDIED

The soils investigated (Table 1) were collected from the top 0 - 30 cm depth of commercial potato fields in Cambridgeshire, Lincolnshire, Nottinghamshire and Shropshire in England between June 2010 and May 2011. These fields were used to evaluate fluensulfone (Nimitz 15G) for management of *G. pallida*. The soils were used after drying for, at least, seven days at a temperature of 20 °C, and sieving through a 2-mm aperture sieve (Endecotts Ltd. London, England). Moss peat was purchased from Clover Peat Products (Dungannon, Northern Ireland), sieved to < 2mm size, and used as an organic amendment. The soils were analysed for pH, residual water content and texture by standard methods (MAFF, 1985; 1986). Soil organic carbon (SOC) content was determined using a LECO sulphur-carbon analyser (LECO®Hazer Grove, Stockport, UK). The soils' water-holding capacity (WHC) was determined using a model 1600 Pressure Plate Extractor (ELE International, Bedfordshire, UK).

CHEMICALS AND REAGENTS

ADAMA Agricultural Solutions Ltd. supplied fluensulfone (a technical-grade of > 95% purity and Nimitz15G®) for the study. Fluensulfone is moderately soluble in water (622 mg/l at 20ºC), has a Log P_{ow} at pH 7.5 of 2.6, and 3.1 x10^{-3} Pa vapour pressure. Stock and working fluensulfone solutions were prepared in 0.01M calcium chloride ($CaCl_2$) (Sigma-Aldrich Limited, UK) in deionised water. Acetonitrile (99.99% purity) and Orthophosphoric acid (85% purity) were purchased from Fisher Scientific Ltd and BDH Laboratory supplies UK Ltd, respectively.

GENERAL METHODS

The sorption of fluensulfone was determined by the batch equilibrium method following the Organization for Economic Co-operation and Development (OECD) guideline 106 (OECD, 2000). Soil suspensions (10 mg soil in 10 ml of 0.01M $CaCl_2$), were prepared and pre-equilibrated by agitating for 16 h on a HS 501 digital reciprocal shaker (IKA®Werke GmbH & Co. KG, Staufen, Germany). All experiments were conducted at a temperature of 20 °C. The suspensions were centrifuged using Eppendorf Centrifuge 5810 (Eppendorf AG, Hamburg, Germany). Supernatants were sampled with 2 ml syringes (BD Plastics Ltd, Sunderland, UK), whenever appropriate, and were sieved through 0.2 μm pore size Polyvinylidene Difluoride (PVDF) syringe filters (GE Healthcare Ltd, Hertfordshire, UK) prior to analysis for fluensulfone. Extracts were analysed immediately or stored at -20 ºC for future analysis. Fluensulfone was quantified by high pressure liquid chromatography (HPLC) analysis following conditions reported by Norshie *et al*., (2017).

DETERMINATION OF FLUENSULFONE SORPTION ISOTHERMS

Sorption isotherms were determined in two separate experiments (Experiment 1 & 2), each conducted three times at four initial concentrations (C_I) of 1.25, 2.50, 5.00 and 7.50 mg/l. Experiment 1 utilised the six original soils whereas Experiment 2 was conducted using Soil HS (See Table 1) following treatments with moss peat at five levels (0.0, 1.2, 2.4, 4.8 and 9.6%). Soil HS was selected for Experiment 2 because it gave the highest scorpion in Experiment 1, and had been used in a pot efficacy experiment. After pre-equilibration, soil suspensions (1/1 w/v) were spiked with an appropriate volume of 50 mg/l stock fluensulfone to give the required C_I. After shaking for 2h (shown in a preliminary experiment to give equilibrium concentration C_E; data not shown), the suspensions were centrifuged at 3000g for 15 min and a 1 ml aliquot was analysed for the C_E. The proportion of fluensulfone sorbed (C_S) was determined as the difference between C_I and C_E. Sorption isotherms were described by fitting the linear form of Freundlich sorption model to C_S and C_E according to the equation in (1), where, K_F and $1/n$ respectively are the Freundlich sorption coefficient and sorption exponent. These parameters were determined by regressing Log C_S against Log C_E, with K_F and $1/n$, respectively, represented by the intercept and slope of the regression equation. Sorption was normalised to soil organic carbon (K_{FOC}) using the equation shown in (2), where OC is the organic carbon contents of soil.

$$\text{Log}\ (C_S) = \text{Log}\ (K_F) + 1/n\ \text{Log}\ (C_E) \qquad (1)$$

$$K_{FOC} = K_F \times 100\ /\ \text{OC} \qquad (2)$$

EFFECTS OF GRANULAR FORMULATION ON FLUENSULFONE SORPTION

This experiment was conducted to compare the sorption of technical-grade fluensulfone with Nimitz 15G® as per sorption coefficient (K_D). The experiment was conducted three times utilising the original soils with the exception of Soil GL. After pre-equilibration, the suspensions (1/1 w/v) were either spiked with 1 mL of 50 mg m/l stock fluensulfone or were treated with 34 mg of granules. The samples were shaken for 2, 4, 6, and 8h, when the liquid phase fluensulfone was quantified. Fluensulfone that was sorbed (C_S), as percentage of the C_I, was determined according to equation (3). K_D was calculated by the equation in (4). In addition to the test suspensions, the setup included two additional samples, both without soil, one of 9 ml $CaCl_2$ spiked with 1 mL of 50 mg/l, the other of 10 ml $CaCl_2$ treated with 34 mg of Fluensulfone 15G. These were used to determine solution concentration of fluensulfone within the period of the experiment, and use to correct for sorption estimates.

$$\text{Sorbed fluensulfone (\%)} = [(C_I - C_T)/C_I]*100 \tag{3}$$

$$K_D = C_S/C_E \tag{4}$$

where, C_T is the concentration measured at the respective sampling times.

EXPERIMENTAL DESIGN AND ANALYSIS OF DATA

The experiments were a randomised block design where each cycle of experiment was treated as a replicate. The data was subjected to analysis of variance in GenStat for Windows® V.15 (VSN International Ltd. Hempstead, UK). Differences between means were compared as per Fisher's protected LSD post hoc tests at $P = 0.05$.

Results

SORPTION ISOTHERMS AND SORPTION COEFFICIENTS

Figure 1 shows the isotherms obtained for fluensulfone sorption in the six original soils Fig. 1A) and the peat-amended Soil HS (Fig. 1B). The Freundlich sorption parameters (K_F and $1/n$), the coefficients of determination (r^2) and sorption normalised to Soil OC (K_{FOC}) are presented in Table 2. The isotherms for the original and peat-treated soils were nonlinear S-type ($1/n > 1$) according to Giles' classification (Giles, 1960). The K_F was lowest in Soil MN and highest in Soil HS ($P < 0.001$). A similar trend was observed for the parameter K_{FOC}. A positive correlation ($r^2 = 0.907$; $P = 0.013$) was found between K_F and soil OC (Table 3). Good, but non-significant correlations were, also, seen between K_F and soil clay ($r^2 = 0.554$; $P = 0.256$) and soil pH ($r^2 = - 0.744$; $P = 0.09$). The peat amendments applied to Soil HS had affected parameters K_F and $1/n$ (Table 2), both of which, correlated positively with Soil OC but significantly only ($r^2 = 0.88$; $P = 0.05$) for the technical-grade (Table 4). $1/n$ was inversely, but not significantly ($r^2 = -0.777$; $P = 0.123$), related to the amendments with the *S*-type isotherm, describing sorption in the original Soil HS (moss peat at 0.0%), being transformed to an L-type isotherm ($1/n <1$) at the 2.4, 4.8 and 9.6% amendments (Table 2). Sorption in the original Soil SH increased by 1.4, 2.6, 3.4 and 3.8-fold, respectively, at the 1.2, 2.4, 4.8, and 9.6% peat treatments.

EFFECTS OF SOIL AMENDMENTS WITH MOSS PEAT ON FLUENSULFONE SORPTION

The peat treatments did not affect the kinetics of fluensulfone sorption in Soil HS (Fig. 2); sorption was fast and reached equilibrium within 2h, and is consistent with preliminary test results (Data not shown). The K_Ds are plotted against the peat treatments in Fig. 3; significantly positive correlations were found between K_D for both formulations of the product and the treatments ($r^2 = 0.752$; $P = 0.036$ for K_DT and $r^2 = 0.996$; $P < 0.001$ for K_DG). Sorption across the soils was significantly greater ($P < 0.001$) after the technical-grade than Nimitz 15G.

EFFECTS OF GRANULAR FORMULATION ON FLUENSULFONE SORPTION

Figure 4 shows sorption kinetics over an eight-hour period for the the technical-grade and Nimitz 15G. Sorption of both forms followed similar kinetics, reaching equilibrium within 2h. This was in agreement with preliminary test results (Data not shown). Sorption after the formulation was lower ($P < 0.001$) than the technical-grade. The sorption estimates were corrected, accordingly, for these differences. The K_D values obtained are presented in Fig. 5. Regardless of soil type, the K_D was higher ($P < 0.001$) for the technical-grade form (Mean K_D = 1.37 mg/kg soil) than the formulation (Mean K_D = 0.32 mg/kg soil). The soil effects were, also, highly significant ($P <$ 0.001); K_D was lowest in Soil MN and highest in Soil WL. Positive correlations were found between K_D and soil OC (Table 4), but marginally significant just after the technical grade form ($r^2 = 0.88$; $P = 0.05$).

Discussion

The results show a two-phase uptake of fluensulfone, with sorption occurring more within the first two hours of making contact with the soils. This would imply a rapid removal from solution, in which case, sorption may become a limiting factor to availability in the soil. On the other hand, this rapid uptake could result in a greater retention of fluensulfone in the soil and could guard against leaching and degradation. Two-phase sorption has been frequently reported in the literature (Boesten, 1990; Ball & Roberts, 1991). The initially fast phase has been ascribed to adsorption processes (Cancela *et al*., 1990), and the slower phase is thought to be due to slow intra-particle diffusion of molecules (White *et al*., 1986). If sorption of fluensulfone on the soils were by adsorption, which accumulates molecules at the soil-liquid interface (Huang *et al*., 1996), then even when sorbed, fluensulfone may be easily accessible to percolating soil water, and perhaps, be readily available for distribution in the soil. In this case, an adsorption driven sorption could concentrate fluensulfone around soil particles thereby exposing second-stage *G. pallida* juveniles to a high dosage. On the other hand, however, greater availability to soil water may suggest vulnerability to leaching agents. Furthermore, it has been suggested (Barbercheck & Duncan, 2004) that aerobic organisms and activity are, mostly, associated with outer surfaces of soil particles. If fluensulfone is predominantly adsorbed, it could as well be that its persistence may be shortened by enhanced availability to microbial degraders.

Sorption of molecules, exhibiting low water solubility, has been suggested to occur mainly by hydrophobic partitioning into soil organic matter (Koenning & Sipes, 1998). This often, yields linear isotherms (Scow & Johnson, 1997). Even though the sorption of fluensulfone depended mainly on the soil organic matter, the isotherms observed were essentially nonlinear ($1/n \neq 1$). This may indicate that the sorption observed was not exclusively influenced by hydrophobic partitioning. An S-type isotherm denotes more rapid sorption as solute concentration increases (Murphy *et al*., 1990), and is characteristic of sorption of organic compounds onto a surface with low sorbing potential (Chiou *et al*., 1998). This condition typically relates to the adsorption of organic compounds onto clay surfaces (Delle Site, 2001; Sposito, 1984). Therefore, the involvement of soil clay in the sorption of fluensulfone is likely. The transformation of the S-type isotherm, describing sorption in the original soil HS, to an L-type isotherm following the peat treatments of this soil provided even greater evidence of uptake by the soil's clay fraction. It is possible that the addition of peat may have had a diminishing effect on the contribution of clay to the sorption process; this could be expected as the proportion of clay in the soils is diluted with the increasing addition of peat. The suggestion, therefore, is that sorption of fluensulfone may be due to hydrophobic partitioning, but also by adsorption onto the mineral fraction, mainly clay.

The sorption of fluensulfone varied widely between the original soils, but was generally, low across the soils. This could mean low sorbing potentials for fluensulfone, at least in the soil studied here. The correlation between fluensulfone sorption and the soils' organic matter content highlights the importance of the molecules hydrophobicity in its affinity for soil. Inferences from the selected properties would suggest moderate hydrophobicity, and could relate to the low sorption across the soils. Indeed, treating Soil HS with peat significantly enhanced this soil's capacity for sorbing fluensulfone. Even then, it is worth noting that the increases in sorption did not commensurate with the levels of the peat treatment, and was a further indication of potentially low affinity of fluensulfone for the soils. Being lowly sorbed implies that substantial proportions of fluensulfone would partition to the soil liquid phase for distribution and nematicidal activities. Furthermore, the generally low K_{FOC} indicates that fluensulfone may be mobile in the soil, and as such, may be highly dispersible in percolating soil water. The low sorption of fluensulfone in soil indicates that it has greater potential for controlling *G. pallida* in a wide range of soils.

The difference in sorption of the technical-grade and the granular product is attributable to limited availability of fluensulfone in the solution for uptake. Indeed, a granular formulation releases active substances gradually (Kile *et al*., 1999) and, was evident for Nimitz 15G (Norshie *et al*., 2017). The lower sorption exhibited here would suggest that studies involving the technical-grade could over-estimate sorption in potato beds receiving a treatment of this formulation. Coupling effects of limited availability and fast sorption further suggest that low soil solution concentrations could be expected when Nimitz 15G is applied to potato beds. Furthermore, the diminishing effect on sorption implies plausible limiting effects on degradation, as well as leaching of the molecules in potato beds.

The results have shown low sorption of fluensulfone in a range of soils, suggesting that the nematicide will be readily available in the soil's aqueous phase for effective control of *G. pallida*. These results, however, indicate that fluensulfone may be easily leached. Even though this highlights environmental consequences, our study on persistence in field soil (Norshie *et al*., 2017) indicated that it may not pose a hazard to the environment.

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