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Chemistry of organic matter in some New Zealand soils: correlation with pesticide sorption

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ABSTRACT

Soil organic matter (SOM) regulates retention behaviour of pesticides in terrestrial environments. It is generally assumed that soils have same reactivity for pesticides per unit mass of organic C. However, the chemical composition of the organic constituents may differ among soils from various agroecological regions. Studies were performed to probe variations in the chemical composition of SOM using solid-state CPMAS ^{13}C NMR technique in a diverse range of New Zealand soils. Carbon chemistry of the soil samples revealed differences in the composition of organic matter in soils from different regions. Generally, N- and O-alkyls and acetals dominated in soils, while alkyl C was the second and aromatic C the third quantitatively most important C types seen by ^{13}C NMR. Overall, proportion of O-alkyl C in the soils varied from 23.3 to 59.5%, alkyl C from 18.4 to 46.6% and aromatic C ranged from 8.8 to 22.4%. Carbonyls were the least abundant group. Our recent studies on the role of the chemistry of SOM in retention propensities for pesticides in soils from various regions demonstrated markedly different sorption affinities for pesticides. Therefore dissimilarities in the chemical nature of SOM need to be considered while estimating sorption capacities of soils for xenobiotics.

INTRODUCTION

Soil organic matter (SOM) is one of the most important components of soil matrix. It dictates soil health and performs a variety of functions. It also plays a critical role with respect to interactions with organic contaminants (Koskinen & Harper, 1990). A clear understanding and quantifying sorption of pesticides onto the soils is essential for predicting their fate and transport in the soil environment. Various models are increasingly used to make these predictions (e.g. Close *et al.*, 1998, 1999; Ma *et al.*, 2000); however, these models do not take into consideration the chemistry of SOM and its role in pesticide interactions. There is a large body of evidence indicating that partition coefficients of non-ionic pesticides is directly correlated to the quantity of SOM; only recent studies have revealed the importance of the chemical nature of SOM with regard to the sorption of pesticides (Ahmad & Kookana, 2002).

The nature and properties of soils are regulated by various soil-forming factors such as parent material, climate, vegetation and time (Buol *et al.*, 1989). As these factors vary widely among regions, soils from various regions may vary correspondingly in their properties. New Zealand has a wide array of soils with relatively high organic matter (>5%

in most of the soils) with an average in the vicinity of 10 to 12% (Burney *et al.*, 1975). A number of soils have been derived from volcanic materials and contain high proportion of allophanic clays, and hence differ from soils of many overseas countries. Vegetation type, nature and magnitude of decomposition process and mineral constituents of the soils are believed to primarily control the quality of the functional groups of the SOM.

Carbon-13 cross polarisation magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy is an important, non-destructive technique that is being commonly utilised to identify the chemical structure of SOM (Skjemstad *et al.*, 1997; Mather *et al.*, 2000). The major advantage of this technique is that it does not alter chemical composition of organic matter and allows probing of the chemistry of SOM within whole soil samples, thereby supplying information on the interactions of organic pollutants with the humic substances (Ahmad *et al.*, 2001).

As information on the chemical nature of SOM is lacking for New Zealand soils, we conducted studies with the objectives to determine the type and relative areas of functional groups (chemical shift regions) using ^{13}C NMR in a wide range of soils occurring in North and South Islands of New Zealand and to assess the relationships of sorption of non-ionic pesticides with the chemical nature of SOM.

MATERIALS AND METHODS

Fifty soil samples (0 – 7.5 cm) from various agro-ecological zones of New Zealand were used in this study. Locations of sampling sites are shown in the map (Figure 1). The soils used were chosen to represent a wide range of soil characteristics. Physico-chemical characterisation of the samples was performed using standard methods. Some of the physical and chemical properties of the samples are presented in Table 1.

Table 1. Range of some general characteristics of the soils studied

Soil Characteristics	North Island (31 soils)	South Island (19 soils)
pH (1: 2 H_2O)	4.9 – 6.9	4.4 – 5.9
Organic C (g kg^{-1})	20 – 276	26 – 419
Clay (g kg^{-1})	80 – 660	20 – 480
Silt (g kg^{-1})	90 – 550	20 – 660
Sand (g kg^{-1})	10 – 700	20 – 990
Soil Type	18 ash	1 ash
	8 sedimentary	16 sedimentary
	3 pumice	2 peat
	2 peat	

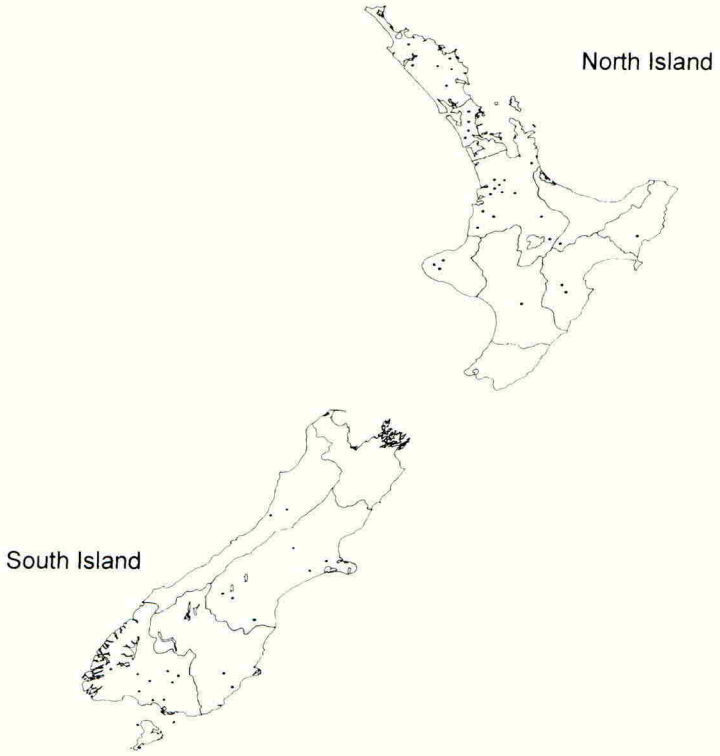

Figure 1. Map of New Zealand showing sampling sites

Prior to determining their NMR spectra, the soil samples were repeatedly washed with 2% hydrofluoric acid to remove paramagnetic materials and freeze-dried. Solid-state ^{13}C CPMAS NMR measurements were performed at magic angle spinning rates of 5 kHz, employing a Bruker Avance 200 MHz Digital spectrometer with a 7 mm probe, operating at 50.33 MHz for ^{13}C. Spectra were acquired using 1 ms contact time, 1 s recycle time, and 5000 scans. The NMR spectra were divided into the following chemical shift regions: 0–45 ppm, alkyl C (C-H); 45–110 ppm, O-alkyl C (C-O); 110–140 ppm, aryl C (Ar); 140–165 ppm, O-aryl C (Ar-O); 165–190 ppm, carboxyl C (C=O) and 190–220 ppm, aldehyde/ketone C (ketone). The proportional contribution of these types of C was determined by integration of the spectra (Inbar & Hadar, 1989).

RESULTS AND DISCUSSION

Solid-state ^{13}C NMR spectra of selected soils are presented in Figure 2. For simplicity, the total peak area was regarded as representing total soil organic C in the sample and refer to the various regions of the spectrum as percentages of the total C. An examination of the spectra revealed several chemical structural variations among the soils collected from different regions of North and South islands of New Zealand. Generally, C resonating in the 45–110 ppm region of the spectra (N- and O-alkyls and acetals) dominated in soils except N14, while alkyl C was the second and aromatic C (aryl + O-aryl) the third quantitatively most important C type seen by the ^{13}C NMR. The abundance of O-alkyls is

likely due to the large concentration of carbohydrates from simple sugars to starches and cellulose in majority of soils under pasture. The NMR spectrum of one soil from North Island (N14) was conspicuously different from the other soils studied. It showed very strong signal for aliphatic C or carbohydrates, which might be due to *in situ* enrichment as a result of the selective decomposition of carbohydrates. Overall, the proportion of O-alkyl C in the soils varied from 23.3 to 59.5%, alkyl C from 18.4 to 46.6% and the aromatic C (aryl+O-aryl) ranged from 8.8 to 22.4%. The carbonyls were always the least abundant group as revealed by the NMR. These variations in the composition may be ascribed to heterogeneity of organic materials found in soils in terms of their source, parent materials, degree of decomposition and environmental factors. The soil samples from North Island showed more aromatic character than those from South Island of New Zealand.

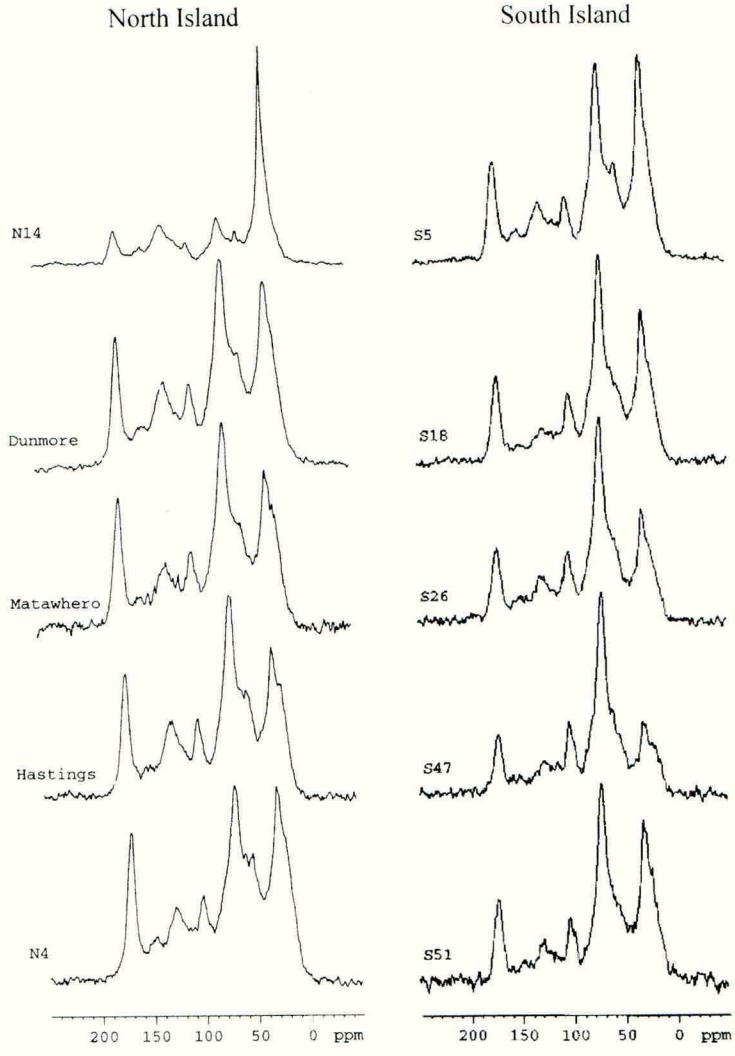

Figure 2. Solid-state ^{13}C NMR spectra of selected soils

The spectral data were analysed by principal component analysis (Figure 3). It displays two sets of information, soil samples (1–31 from North & 32–50 from South Island) and explanatory variables (vectors). Along the first principal component, samples towards the LHS of the plot have above average concentrations of O-alkyl, below-average concentrations of aromatic and O-aromatic and a range of above and below average concentrations of alkyl C. Along the 2nd principal component, soils No 5, 10 & 32 contain higher proportion of aryl component and are very different from other samples.

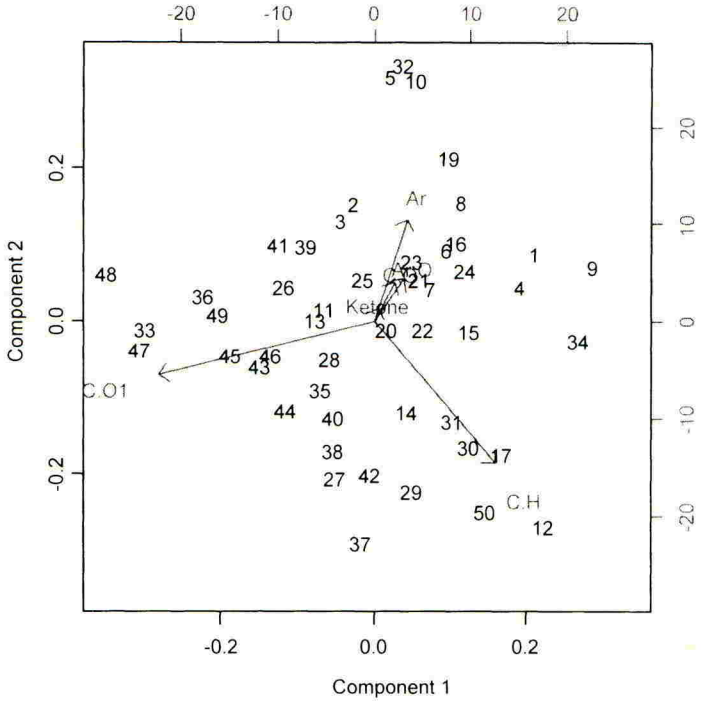

Figure 3. Biplot for principal components 1 and 2 for components of SOM

The information presented above shows that it would be useful to relate the chemical nature of SOM to the sorption affinities of pesticides. However, attention has not been paid to the relationships between various physical and chemical properties of SOM and the behaviour of pesticides in soils. The relationships between the K_{oc} values of two non-ionic compounds (carbaryl and phosalone) and the chemistry of SOM were investigated and found that among various structural components of SOM, the aromatic component had a significant impact on the sorption of both pesticides (Ahmad *et al.*, 2001). Stepwise multiple linear regression also suggested that the K_{oc} values of both pesticides were adequately predicted using aryl C together with carboxyl C ($p<0.001$). Therefore, variations in the chemical composition of the SOM should be considered in predictive models for fate and transport of pesticides and risk assessment. Further studies are being conducted to explore more exhaustively the relationships between the binding affinities of organic contaminants and various structural and molecular components of SOM.

ACKNOWLEDGMENTS

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Analytical support of the DuPont Quality Programme stewardship initiatives in the Nordic Region

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ABSTRACT

The DuPont sulfonylurea herbicides are commonly used for control of broadleaved weeds and grasses in the Nordic region. Their recognized attributes include low relative use rates, low mammalian toxicity and an environmentally friendly profile. The focus of the DuPont Quality Programme is the strict maintenance of product quality and the oversight of product labeling such that measures are in place to ensure safety to users and the environment. As part of this programme, and in order to enhance our ongoing stewardship efforts, we are proactively conducting water-monitoring programmes in three locations in Sweden. Our definitive and highly sensitive multiresidue method for the determination of sulfonylurea herbicides in water has now been successfully transferred to a Swedish private laboratory to support this programme. Minute traces of these products at levels below any thresholds of ecotoxicological concern are occasionally detected. On rare occasions, levels at or near 0.1 µg/L are found, and are attributable to spot contamination. We have also put in place the analytical capabilities for our customers, should there be a need, to have their crop and soil samples analyzed for trace levels of sulfonylureas. The result is increased customer satisfaction and a renewed comfort level with our products.

INTRODUCTION

In 1998, the DuPont Quality Programme was initiated in the Nordic Region to support our customers in the use of our sulfonylurea herbicide products. This programme has four major components: (1) ISO 14001/9000 compliance in our manufacturing facilities; (2) state of the art formulations that are safe and easy for customers to handle; (3) information technology to make product information and MSDS sheets readily available to our customers and (4) water quality. The focus of this paper will be on the water quality component.

Sulfonylurea herbicides are used in Scandinavia for control of grasses and broad-leaved weeds in cereals, potatoes and sugar beets. They are considered to be environmentally safer than most older herbicides due to their low application rates (typically on the order of several grams per hectare), low mammalian toxicity and rapid breakdown in soil, water and plants. However, the concern about pesticides in the environment in the Nordic countries is very high, and many government and private organizations are engaged in water monitoring activities to ensure that pesticides are not present in drinking water above any levels of toxicological or environmental concern. Sulfonylurea herbicides cannot be determined by most of the multiresidue analytical

methods commonly used in water monitoring, because they are not amenable to gas chromatography. Therefore, these compounds are typically not included in most water monitoring programmes. As a result, DuPont is proactively conducting water monitoring at three sites in Sweden that are representative of the agricultural regions where sulfonylurea herbicides are most commonly used.

MATERIALS AND METHODS

Water samples were collected from three sites in Sweden. One site (Vemmenhög, Skåne County) was studied since the programme began in 1998. Two additional sites (Halland and Västergötland Counties) were added in 2002. The sites were chosen so that both surface water and tile-drainage water (at approx. 1 m depth) runoff from multiple farms all focused at a single sampling point. An automated water-sampling device was placed at the focal point of the runoff. The device collected small samples of water twenty times a day and combined them into a container enclosed in a refrigerated compartment. The composite samples were removed from the refrigerator each week, from May through November, so that four or five weekly samples were collected each month. The samples were then frozen until the entire season was collected, and shipped to the analytical laboratory.

Water samples were analyzed for the six DuPont sulfonylurea herbicides used in Swedish agriculture. The compounds determined were tribenuron methyl (Express® Herbicide), thifensulfuron methyl (Harmony® Herbicide), metsulfuron methyl (Ally® Herbicide), flupyrsulfuron methyl (Lexus® Herbicide), triflusulfuron methyl (Safari® Herbicide), and rimsulfuron (Titus® Herbicide). The method that was used for the 1999-2002 programmes is described by Powley (2003). Briefly, the method required adjustment of the water sample pH to 6.5 through addition of ammonium acetate, followed by concentration and cleanup using a Bond Elut ENV® (Varian Corporation, Harbor City, CA, USA) polymer resin solid-phase extraction cartridge. The analytes were eluted with 25 mM ammonium hydroxide in methanol. The eluate was evaporated to dryness and reconstituted in 5 mM ammonium acetate. Analysis was done using reversed-phase liquid chromatography and a triple quadrupole mass spectrometer equipped with an electrospray interface operating in the positive ion mode.

Samples collected in 1998 were analyzed at Covance UK (Harrogate, UK), and the limit of quantitation (LOQ) was 0.01 µg/L. The samples from the 1999 and 2000 programmes were analyzed at DuPont in the USA and from 2001 to the present they were analyzed at AnalyCen Nordic AB Laboratories in Lidköping, Sweden. The LOQ was 0.05 µg/L at both laboratories until 2002, when it was decreased to 0.01 µg/L. Two parent to daughter transitions were monitored for each analyte. The more intense transition was used for quantitation and the ratio of the two transitions was used for confirmatory purposes.

RESULTS AND DISCUSSION

Analytical Method Performance

The analytical method was validated at 0.05 and 0.5 µg/L (LOQ and 10xLOQ). Recovery levels typically ranged from 80 to 110%, with relative standard deviations of approximately 10%. Reagent blanks were run on a regular basis to ensure that contamination was not

occurring. Interferences were very rare, and usually amounted to only 5 to 10% of the LOQ. Interferences were distinguished from contamination and actual detections through the use of the confirmatory parent to daughter ion transition signal. The signal to noise ratio of the least responsive analyte at LOQ was typically 10 to 20, with higher values for the other analytes. Detection limits therefore were typically 0.001 to 0.005 µg/l. Calibration curves were linear, with negligible intercepts.

Results of Water Monitoring Samples

The samples were analyzed for six sulfonylurea herbicides. Rimsulfuron and metsulfuron methyl were never detected in any samples during any of the seasons monitored from 1998 to 2002. Only three isolated detections of triflusulfuron methyl and one detection of flupyrsulfuron methyl have occurred at levels below the LOQ of the analytical method. Thifensulfuron methyl was detected in one sampling at Halland (0.021 µg/L) and one sampling at Västergötland (0.14 µg/L) in 2002 (the first year of monitoring for those sites). These levels are below biological activity thresholds for non-target aquatic plants.

The other analyte, tribenuron methyl, is the most commonly used sulfonylurea herbicide in the Nordic region, used extensively in cereal production. From 1998 to 2002, there were a total of ten detections of tribenuron methyl at the Vemmenhög site, out of a total of approximately 160 samples collected. All detections except one were below the quantitation limit of the analytical method in effect at the time of analysis, which was below any threshold of biological activity as evaluated in ecotoxicological testing with non-target aquatic plants (algae and *Lemna gibba*). (One detection at Vemmenhög in 2002 (0.016 µg/L) was slightly above the quantitation limit of 0.01 µg/L.) Figure 1 shows the distribution over time of the detections for tribenuron methyl in Vemmenhög, for each of the years the programme was run. Tribenuron methyl was detected one time at Halland (0.010 µg/L) and Västergötland (0.077 µg/L) in 2002, which was the first year of monitoring.

Tribenuron methyl applications are made at early post emergence, typically in April or May. Therefore, detections in May and early June usually can be attributed to surface water runoff, direct overspray of the sampling stream, movement via soil cracks or via inspection wells to the tile drainage system. Detections later in the season can be attributed to movement of the herbicide through soil cracks and inspection wells, or via normal movement through the soil profile into tile drainage system and then to the sampling point. In either case, the levels detected were well below those of any ecotoxicological concern. The detections were also isolated, which indicates that there is no constant, long-term exposure of the environment to measurable levels of tribenuron methyl. These results are consistent with those obtained in laboratory and field studies conducted elsewhere in Europe and North America. Although the water sampled in this study is considered surface water from agricultural runoff, as opposed to ground water to be used for drinking water, the levels found are rarely above the EU Drinking Water Directive Level of 0.1 g/L.

After each growing season, the results of the water monitoring programme were communicated to the growers in the area. The number of detections, as well as their timing, can be used to improve agricultural practices so that introduction of herbicides into the water can be minimized even further. For example, early season detections were attributed to spraying or cleaning spray tanks too close to wells. Once the wells were covered and behaviour patterns changed, the number of detections in the following years decreased.

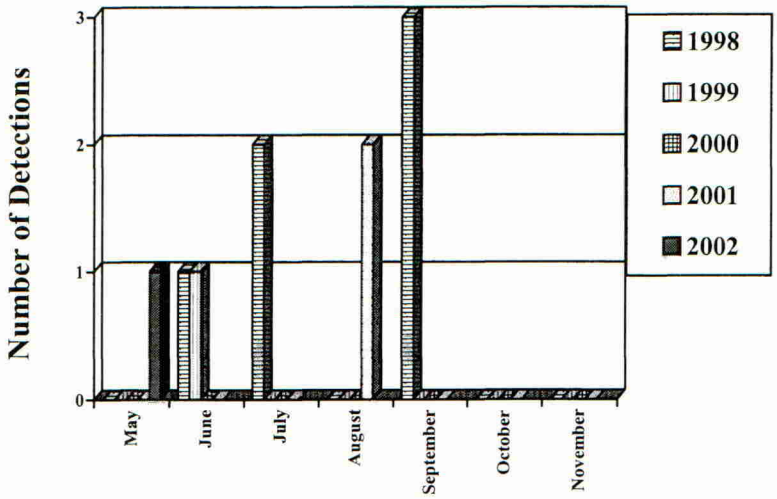

Figure 1. Tribenuron methyl detections at Vemmenhög, Sweden. The total number of detections in each month (4 or 5 samples were collected each month) is represented by each column bar.

As an extension of this programme, we have also transferred methods for determination of sulfonylurea herbicides in crops (cereals, sugar beets and potatoes) to AnalyCen Nordic AB. Our customers are now able to submit samples of their produce to this laboratory, in order to certify that their crops contain non-detectable residues of sulfonylurea herbicides. Since residues of these herbicides are non-detectable in food crops harvested at maturity, they can be certified as pesticide-free and are in compliance with the EU Babyfood Directive. Finally, transfer of a method for determination of sulfonylurea herbicides in soil is in progress. Once this method is in place, our customers will be able to check their soil if there are any concerns about carryover from earlier applications.

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Laboratory studies on flumioxazin sorption and persistence in soil

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ABSTRACT

The adsorption and desorption of flumioxazin was tested on seven soils. The half-life ($t_{1/2}$) of flumioxazin in Greenville sandy clay loam and Tifton loamy sand soils were tested at 15 and 25°C. Adsorption isotherms for all soils had K_f values that ranged from 8.8 to 0.4, with the majority occurring near 1.5. These low K_f values indicated that flumioxazin does not form strong associations with sorbents tested. As clay and organic matter content increased for soils in this experiment, flumioxazin adsorption also increased. With the high correlation of organic matter content and sorption, K_{OC} values were derived for each soil. The K_{OC} values for each soil were similar, indicating that organic matter was the dominant factor affecting adsorption. These results denote that desorption was only marginally slower than adsorption. Therefore, flumioxazin can become readily available in soil solution with the addition of increased soil water content. Results indicated that temperature had little effect on flumioxazin persistence. The $t_{1/2}$ for the Greenville soil was 17.9 and 16.0 d while the Tifton soil was 13.6 and 12.9 d, at 15 and 25°C, respectively. These data correspond to the greater clay content of the Greenville soil (32%) as compared to the Tifton soil (2%).

INTRODUCTION

Flumioxazin is a soil applied *N*-phenyl phthalimide herbicide with a wide spectrum of weed control and a reported low potential for soil carryover to rotational crops such as cotton (*Gossypium hirsutum*), corn (*Zea mays*) and vegetables (Vencill 2002). Currently, limited information exists concerning the environmental fate of flumioxazin. Studies have indicated that flumioxazin has a soil half-life between 11.9 and 17.5 d (Vencill 2002).

Therefore, the objective of this study was to characterize flumioxazin adsorption and desorption in seven soils and the half-life of flumioxazin in two commonly occurring peanut soils. The influence of incubation temperature and soil microbial population on dissipation of flumioxazin was also investigated.

MATERIALS AND METHODS

Adsorption-Desorption: A batch equilibrium technique was used to determine adsorption of flumioxazin to soil. Formulated herbicide (Valor 51 WP) and ^{14}C-flumioxazin [Phenyl-^{14}C, specific activity 12.9 MBq mg^{-1}] was added to 0.01 M $CaCl_2$ solutions to achieve concentrations of 0, 10, 20, 40, and 60 µmol l^{-1}. Ten ml of solution was added to a 2-g soil sample that had been dried and passed through a 1 mm sieve. Samples were placed on a wrist action shaker at 20°C for 24 hours. After shaking, the samples were centrifuged at

4,400 x *g* for five minutes. A 2-ml aliquot of supernatant was removed from each vial and counted for activity using liquid scintillation spectroscopy. Three replications were used and a blank, containing no soil, was included with each run. Preliminary experiments showed that flumioxazin adsorption to centrifuge tubes were negligible (data not shown). The characteristics of the soils used in this experiment are shown in Table 1.

Adsorption kinetics was measured using Greenville sandy clay loam soil with 3 replications. Ten ml of 40 µM flumioxazin was added to each tube and placed on the shaker as described above in batch equilibrium experiment. Samples were removed from the shaker after 1, 6, 12, 24, 48, and 72 hours and centrifuged. Herbicide not in solution was considered soil adsorbed. The distribution of herbicide between the absorbed and solution phases was calculated using the Freundlich equation. Since the Freundlich equation was used to the sorptive relationships, K_f values will be reported. Using the calculated K_f values, K_{OC} was calculated as $K_{OC} = (K_f \div \%OC) \times 100$. Data were expressed as percent of total herbicide absorbed.

Desorption was also measured using Greenville sandy clay loam soil with 3 replications. The samples were prepared as described above in the adsorption section and brought to equilibrium by shaking for 24 hours. The samples were centrifuged and a 2-ml aliquot was removed for flumioxazin quantification by LSS. The remaining $CaCl_2$ solution was poured from each tube and replaced by herbicide free 0.01 M $CaCl_2$ solution. Approximately 90% of total solution was removed at each sample timing (data not shown). This process was repeated after 24, 48, 72, and 96 hours. After 96 hours of desorption, the soil pellet was dried and then combusted in a biological so a mass/balance test could be preformed. Upon conclusion of the experiment, 96% of the total flumioxazin was accounted for. Desorption data were fitted to the Freundlich equation as previously described.

Persistence: Greenville sandy clay loam and Tifton loamy sand soils were gathered from the top 10 cm of cropping areas that were void of previous flumioxazin application. The soils were air dried and passed through a 2 mm sieve. Twenty-five g of dry soil was placed in plastic bottles and capped (Dinelli *et al.*, 2000). One ml of flumioxazin, dissolved in acetonitrile at a concentration of 100 ng ml^{-1}, was added to each bottle. After the solvent had evaporated, the soil sample was mixed for even herbicide distribution. Water was added to each bottle in order to achieve 70% of field capacity. The samples were incubated at either 15 or 25°C for 0, 0.25, 0.5, 1, 1.5, 2, 4, 8, 16, and 32 d in the dark. All remaining bottles were opened after 8 and 16 d of incubation to ensure that the environment remained aerobic. Samples were stored at -20°C until extraction.

To determine the influence of microbial degradation on the persistence of flumioxazin, the Tifton ls soil was heat treated for 48 h at 90 °C to reduce microbial populations. Flumioxazin was recovered from each soil using accelerated solvent extraction (ASE) in acetonitrile (Vencill & Ferrell, 2003). The samples were then reconstituted with 1 ml of acetontirile and flumioxazin concentration was analyzed using gas chromatography (HP 6890) coupled with mass spectroscopy (HP 5973). Half-lives($t_{1/2}$) for each temperature were determined, as in Lehmann et al. (1993). Rate constants were determined from the slope of the linear regression line. The experiment was a completely randomized design with three replications.

RESULTS AND DISCUSSION

Flumioxazin Adsorption. Sorption non-linearity is common when isotherms are developed using the Frendulich equation to plot *S* on *C* (Reddy & Locke, 1998). However, non-linearity was not observed within the adsorption experiments presented here. This was shown by the fact that *n* values were near unity and r^2 values were ≥0.97 (Table 1). The isotherms were linear because the flumioxazin rates employed in this study did not cover a concentration range greater than 2 orders of magnitude (Wauchope *et al.*, 2002). Large concentration ranges result in non-linearity (even at low concentrations), because a finite number of sorption sites exist at any given energy level (Akratanakul *et al.*, 1983).

Table 1. Sorption parameter coefficients for flumioxazin adsorption to soil.

	Soil Properties						Adsorption			
Series	pH	CEC	% OC	% sand	% silt	% clay	K_f	1/n	pH	K_{oc}
Lanton silt loam	5.8	13.8	5.3	16	60	24	8.8	1.5	0.99	1.6
Duval sandy loam	4.7	3.2	0.8				1.1	0.9	0.99	1.4
Brownfield fine sand	7.3	5.3	0.2				0.4	0.8	0.97	2.0
Greenville clay loam	6.0	7.6	2.6	40	25	34	3.8	1.0	0.97	1.5
Dothan sandy loam	5.1	3.4	0.9	85	12	3	1.5	1.0	0.97	1.6
Cecil sandy loam	5.4	4.9	2.6	62	20	18	3.4	1.1	0.99	1.3
Tifton loamy sand	6.1	4.3	1.6	94	4	2	0.7	0.8	0.99	1.2

Herbicide distribution toward the solid phase was quite low for many of the soils tested. K_f values ranged from 8.8 to 0.4 (Table 1). The Greenville and Lanton soils had the highest degree of sorption with K_f values of 3.8 and 8.8, respectively. Conversely, the Brownfield series was the least adsorptive (K_f = 0.4), due to very low clay and organic matter content. A pairwise correlation procedure was conducted based on these findings to calculate the degree of correlation each soil parameter exhibited with respect to K_f. All parameters were statistically correlated to K_f except or soil pH.

The positive correlation of organic matter to adsorption has been well documented in the review by Wauchope *et al.* (2002). Therefore, the highly significant correlation of K_f and soil organic carbon percentage (p=0.001) was expected. The correlation between soil organic matter and sorption led to the calculation of K_{OC} values for each soil. Previous research has shown that K_{OC} data has proven most useful and reliable for non-ionic compounds with low water solubility (Harper, 1994). It is observed from Table 1 that the K_{OC} for each soil is very similar. This implies that soil organic matter is the principle adsorptive surface in the soil matrix (Sheng *et al.*, 2001). A high degree of sorption by soil organic matter most notably occurs for compounds with low water solubility. This is because organic matter is generally more hydrophobic that the surfaces of silicate clays, allowing hydrophobic molecules to readily partition into the organic phase (Piccolo, 1994). Therefore, flumioxazin, with a water solubility of 1.78 mg l^{-1} (Vencill, 2002), would be expected to preferentially adsorb to the organic matter fraction of soil.

Previous research has demonstrated that K_f values can vary as much a 15-fold, depending on the interaction between certain herbicides and soil properties (Dyson *et al.* 2002; Clay & Koskinen, 1990). For these experiments, adsorption of flumioxazin varied by approximately 22-fold across a wide range of soils with different textural properties. However, considering the soils that dominate the peanut growing regions of the southern U.S., the calculated K_f value for flumioxazin was near 1.5. This indicates that flumioxazin possesses near equal affinity for the sorbed or solution phases. Therefore, flumioxazin does not bind tightly to the soil matrix and can be readily removed from adsorption sites by simply increasing soil water. By increasing the quantity of flumioxazin in solution, there is greater probability for injury to occur to non-target species, such as the crop plants.

Sorption of flumioxazin to a Greenville clay loam soil was very rapid within the first 1 hour time segment. Approximately 72% of total herbicide was adsorbed after 1 hour. Adsorption continued to increase with time until reaching 78% after 72 hours of continuous shaking. By comparing the standard errors of the means (approximately 0.6%), this increase in adsorption with time was significant. This time-induced increase in adsorption is indicative of herbicide adsorption kinetics experiments (Reddy & Locke, 1998). Increased adsorption, after the initial rapid phase, could be due to the exposure of additional sorption sites as a result of frictional wear from the shaking process. Regardless, flumioxazin adsorption is near instantaneous with respect to time, but increased shaking time increased sorption by as much as 6% after 71 hours.

Flumioxazin Desorption: Herbicide desorption experiments often yield hysteric results (Reddy & Locke, 1998). Hysteresis is when pesticide desorption from soil is less than predicted by adsorption. Thus, pesticide desorption occurs at a different rate than adsorption. The presence of hysteresis often implies that a herbicide is interacting strongly with soil surfaces.

Desorption of flumioxazin was measured on a Greenville clay loam soil. Desorption data were subjected to the Frendulich equation and rate of desorption was indicated by the slope of the line (Figure 1).

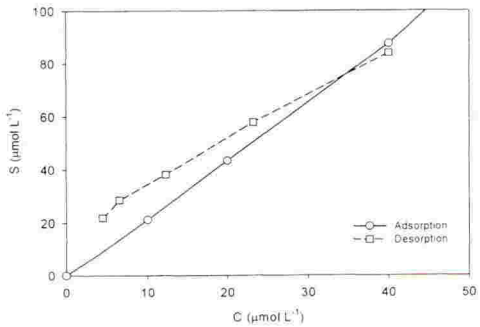

Figure 1. Adsorption isotherm (solid line and closed symbols) and desorption isotherm (hatched line with open symbols) of a Greenville clay loam soil. The desorption isotherm had an initial concentration of 40 µmol l^{-1}. The r^2 for each isotherm was >0.98.

Comparing flumioxazin desorption and adsorption, it was determined that the rates of these two processes were similar, with hysteric effects being observed. Flumioxazin adsorption

and desorption processes occurred at similar rates, reaching equilibrium in soil solution quickly without delays taking place due to surface charge interaction. Therefore, flumioxazin does not form strong adsorptive associations with soil colloids. This occurrence of similar adsorption and desorption rates, as well as the low K_d values as demonstrated by the adsorption isotherms, suggest that flumioxazin and would be readily available in soil solution after significant rainfall. These characteristics would potentially make flumioxazin subject to downward movement in the soil profile, absorption by plant roots, and degradation by soil microbes. However, additional experiments detailing the persistence and mobility of flumioxazin would be required to fully substantiate this hypothesis.

Data presented here indicate that flumioxazin is not tightly adsorbed to soil colloids. K_d values were narrow, 2.1 to 0.9, although the soils used for experimentation varied with respected to percent clay and organic matter content. Moreover, adsorption of flumioxazin is dependent on percent clay and sand content of the soil, rather than percent organic matter. The adsorption and desorption properties of flumioxazin make it likely to be available in large quantities for plant uptake and microbial degradation upon each addition of water to the soil.

Flumioxazin Persistence: The results of the flumioxazin dissipation study are summarized in Figure 2. Incubation temperature had little impact on the dissipation rate of flumioxazin in either soil.

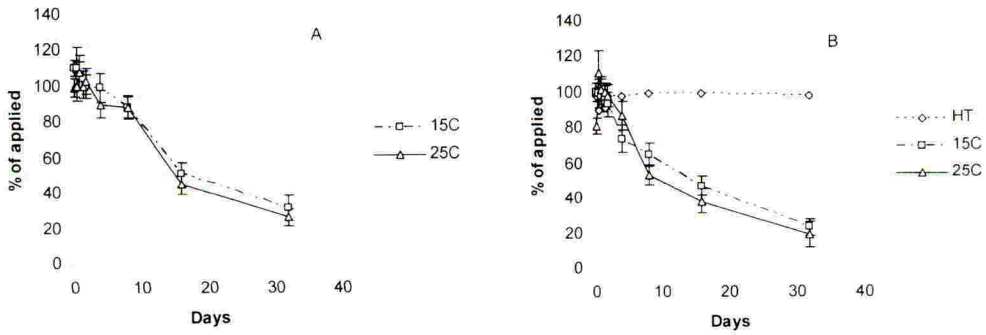

Figure 2. Dissipation of flumioxazin from (A) Greenville sandy clay loam and (B) Tifton loamy sand over 32 d at 15 and 25 °C. Heat treatment (HT) was used to reduce microbe populations within the soils sample. Error bars represent the standard error of the mean.

However, soil microbes were observed to be the predominant factor influencing flumioxazin dissipation. This was indicated by observing the lack of flumioxazin dissipation in the HT soil. After 16 d, the HT soil retained near 99% of the originally added flumioxazin. At 70% field capacity, <1% hydrolysis had taken place after 16 d of dark incubation. Therefore, when soil microbe populations are reduced, flumioxazin dissipation rates are greatly retarded.

The $t_{1/2}$ for the Greenville scl soil was 17.9 and 16.0 d, while the Tifton ls was 13.6 and 12.9 d, at 15 and 25 °C, respectively. Temperature had a relatively small impact on flumioxazin

degradation. For herbicides with soil half-lives greater than 100 days, such as atrazine and flumetsulam, increases in temperature can have dramatic effects on degradation rates (Lehman *et al.*, 1993). However, herbicides with half-lives from 10 to 20 days, such as metolachlor, degradation rates have been shown to be less effected by temperature. Therefore, flumioxazin, with half-lives between 12 and 17 days, was not expected to be greatly influenced by incubation temperature.

The half-live data for flumioxazin was similar to the previously published dissipation times of 11.9 to 17.5 d (Vencill, 2002). The reason for the greater $t_{1/2}$ values with the Greenville scl soil was likely influenced by the increased adsorption by this soil. Previous reports have stated that pesticides that are sorbed to soil surfaces are not available for degradation by soil microorganisms. Flumioxazin sorption in the Greenville scl was greater than that of the Tifton ls (K_d of 3.8 as compared to 0.7); however, the $t_{1/2}$ was only increased by approximately 4 d.

The laboratory persistence of flumioxazin was found to be relatively short, regardless of soil textural properties. However, soil persistence was positively related to the adsorption coefficient of the given soil. Although flumioxazin dissipation in soil occurred relatively quickly, mineralization rates were much lower. Therefore, the processes of microbial degradation and mineralization of flumioxazin are greatly separated in time.

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Validation of analytical methods for the determination of agrochemical residues in air, using a simulated sampling technique

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ABSTRACT

Analytical methods for three test compounds ('A', 'B' and 'C') were validated using the described procedure. Front portions of air traps were fortified with test compound, in quintuplicate at discrete levels, equivalent to air concentrations ranging from the limit of quantification (LOQ), to the highest air concentration to be monitored. Air was passed through the traps at a flow rate of *ca.* 1 ml/minute continuously, for six hours, (i.e. a total volume of *ca.* 360 L air), with the section of fortified adsorbent facing the air flow. Simulated air sampling was carried out with air at ambient laboratory temperature and humidity and with air at a temperature of 35°C and a relative humidity of at least 80%. Temperature, relative humidity and air flow were monitored at intervals during the six-hour sampling period. At the end of the sampling period, the front and rear sections of each trap were removed and analysed separately to check for breakthrough into the rear trap material. The amount of test compound recovered from fortified traps, after simulated sampling, was used as a measure of the validity of the method. Results for each test compound showed acceptable mean recovery and precision at each validation level, under both ambient and elevated sampling conditions. Breakthrough to the rear traps was observed only under the elevated temperature and humidity conditions and was considered acceptable.

INTRODUCTION

In the European Union, validation of residue analytical methods should comply with the requirements of Council Directive 91/414/EEC and associated guidance documents (European Commission, 1996; SANCO 2000a, b). These documents specify the regulatory requirements for the validation of air sampling methods. Analytical methods should be suitable for determining spray drifts, dusts and vapour emissions as well as gaseous residues. Consideration should be given to the expected concentrations in air, retention of the analyte and the time period required for sampling.

The proposed limit of quantification (LOQ) should take into account the relevant human and ecotoxicolgical based limit values, or exposure levels, and should be sufficient to quantify exposures well below the No Observed Effect Level (NOEL), divided by an appropriate safety factor.

Limit of quantification (LOQ)

The proposed LOQ for each test compound was calculated from the following equation:

$$LOQ = \frac{AOEL \times SF \times BW \times 1000}{V} \mu g/m^3 \text{ air}$$

Where: AOEL = Acceptable Operator Exposure Level (inhalative or systemic; mg/kg bw/day)
SF = safety factor (maximum 0.1)
BW = body weight (60 kg)
V = volume of air intake (20 m^3 per day)

The inhalative Acceptable Operator Exposure Level ($AOEL_{inhalative}$) was substituted by the systemic Acceptable Operator Exposure Level ($AOEL_{systemic}$), where the former was unavailable. In the case that both AOEL values were not available, the proposed or set Acceptable Daily Intake (ADI) value was used instead.

Table 1. Calculated Limit of Quantification (LOQ) values

Compound	AOEL (mg/kg bw/day)	ADI (mg/kg bw/day)	Safety Factor	LOQ calculated ($\mu g/m^3$)
'A'	0.7	-	0.001	2.1
'B'	0.05	-	0.01	1.5
'C'	n/a	0.024	0.01	0.72

NOTE: n/a = not available

The simulated air sampling technique described in this paper was designed to allow the validation of analytical methods for the determination of agrochemical residues in air to the EU regulatory guidelines.

MATERIALS AND METHODS

Analytical method validation

A typical procedure for the validation of a method of analysis for an agrochemical in air consisted of the following stages:

- Fortification of the test compound onto air sampling tubes and flushing of the tubes with air under ambient and elevated temperature / humidity conditions.
- Solvent extraction of the test compound from the trap material.
- Quantification by gas chromatography with electron capture detection (GC/ECD) or HPLC with tandem mass spectrometric detection (LC/MS/MS), as appropriate.

Extractability and breakthrough during simulated air sampling from air traps

The test compound was fortified onto prepared air traps in quintuplicate. The air traps used were of two types: Perkin-Elmer air sampling tubes A15012 (8.9 cm x 0.5 cm id, each hand-packed with 450 mg ultra-clean XAD-2 resin) or commercially available pre-packed MTO-ORBO 613 air sampling tubes containing XAD-4 resin (80 mg and 40 mg). The type of tube and packing material selected was dependent on the nature of the test compound and

suitability was determined during method development. Test compounds 'A' and 'B' required the use of Perkin-Elmer air sampling tubes A15012 (XAD-2 resin) and test compound 'C' required the use of MTO-ORBO 613 air sampling tubes (XAD-4 resin).

Front portions of air traps were fortified in quintuplicate, at each validation level (equivalent to the calculated LOQ and 10x LOQ, with 100x LOQ if required), together with two unfortified control traps. Air was passed through the traps at a flow rate of *ca.* 1 ml/minute continuously, for 6 hours, (i.e. a total volume of *ca.* 360 litre air), with the section of fortified adsorbent facing the air flow.

Simulated air sampling was carried out at with air at ambient laboratory temperature and humidity and with air at a temperature of 35°C and a relative humidity of at least 80%. A laboratory incubator of sufficient size to ensure adequate airflow through each of the air traps was used to generate air at the elevated conditions of temperature and humidity. Five air sampling tubes were fortified at each validation level for each set of sampling conditions.

Temperature, relative humidity and airflow were monitored at intervals during the six-hour sampling period. At the end of the sampling period, the front and rear (if present) portions of each trap were removed and analysed separately to check for breakthrough into the rear trap material. Breakthrough was assessed at 10x LOQ and 100x LOQ validation levels only and was considered acceptable if the amount in the rear portion was <10% of the amount of test compound added.

Extraction from the air traps and quantification of the test compounds

Each test compound was extracted from the trap material by hand-shaking, or ultrasonication at room temperature, in a solvent appropriate to the solubility of the test compound.

Test compound 'A' was extracted with 3x 10 ml of methanol in an ultrasonic bath for 10 minutes, the extracts were filtered through glass wool and the combined volume reduced to 3ml by evaporation, using nitrogen convection at 40°C. 7 ml of 0.1% $^{v}/_{v}$ formic acid in water was added and the mixed solution was analysed by LC/MS/MS. Test compound 'B' was extracted with 2x 5 ml of acetonitrile:water (50:50 $^{v}/_{v}$) by hand-shaking for *ca.* 1 minute on each occasion. The extracts were filtered through glass wool, combined and analysed by LC/MS/MS. Test compound 'C' was extracted with 2x 5 ml hexane by hand-shaking for *ca.* 1 minute followed by ultrasonication. The extracts were filtered through glass wool, combined and analysed by GC/ECD.

DISCUSSION

Validation of the analytical method for each test compound included consideration of the following criteria.

Linearity

In order to establish the linearity of response of the analytical chromatographic systems to the test compound, at least six standard solutions of increasing concentration were prepared. The lowest concentration was equivalent to <50% of the concentration of a sample at the limit of

quantification (LOQ). The solutions were injected into the chromatographs in random order, and a concentration/response curve was prepared.

Specificity

The ability of the method to distinguish between the test compound and other substances present in the control samples was evaluated. Components present in control samples were considered not to interfere with analysis if they were present at levels ≤ 30% of the limit of quantification. It was observed that analysis by HPLC/UV was prone to contamination from materials washed from the trap resins during solvent extraction and therefore an alternative means of detection was required (e.g. LC/MS/MS).

Precision

The repeatability of the method was demonstrated by analysing each validation level in quintuplicate. Precision, as relative standard deviation (RSD %), was determined at each validation level and overall and was considered acceptable if ≤20% RSD.

Recovery

Recovery of the test compound from the air traps, fortified at each validation level, was determined in quintuplicate. In addition, two control traps were extracted and analysed. Mean recovery at each validation level and overall was considered acceptable if it was within the range 70% to 110% of the amount of test compound fortified.

Method confirmation

Test compounds 'A' and 'B' were analysed using LC/MS/MS. This technique was considered sufficiently selective and sensitive as to be inherently self-confirmatory and an additional confirmatory method was not required for these test compounds. Test compound 'C' was analysed by gas chromatography with electron capture detection (GC/ECD), with separate confirmation by gas chromatography and mass spectroscopic detection (GC/MS).

Validation results for three test compounds

Linearity of response was established for each test compound, with the lowest calibration standard solution equivalent to <50% of LOQ in each case. Components detected in the control traps were <30% of LOQ, for each test compound, under each set of sampling conditions and specificity was thereby demonstrated for each test compound.

Table 2. Validation results in air at ambient temperature & humidity

Test compound	Validation level (mg/kg)	No. of replicates	Mean recovery (%)	Precision (% RSD)	Breakthrough (%)
'A'	LOQ	5	94	5.5	n/a
	10x LOQ	5	90	3.4	0.0
	Overall	10	92	4.9	n/a
'B'	LOQ	5	98	8.2	n/a
	10x LOQ	5	94	1.5	0.0
	Overall	10	96	6.0	n/a
'C'	LOQ	5	99	7.2	n/a
	10x LOQ	5	110	6.1	0.0
	100 x LOQ	10	95	20.1	0.0
	Overall	20	100	15.4	n/a

NOTE: n/a = not applicable

Results for the three test compounds (Tables 2 & 3) showed acceptable mean recovery and precision, at each validation level, under both ambient and elevated sampling conditions, with a single close exception. Compound 'C', at 100x LOQ, gave a precision of 20.1% RSD, which was just in excess of the acceptance criterion of 20% RSD. However, validation for test compound 'C' was considered acceptable, as overall precision and precision at LOQ and 10x LOQ were < 20% RSD.

Breakthrough to the secondary traps was <10% in each case and was considered acceptable.

Table 3. Validation results in air at elevated temperature & humidity

Test compound	Validation level (mg/kg)	No. of replicates	Mean recovery (%)	Precision (% RSD)	Breakthrough (%)
'A'	LOQ	5	83	2.5	n/a
	10x LOQ	5	84	1.4	6.4
	Overall	10	84	2.0	n/a
'B'	LOQ	5	87	9.6	n/a
	10x LOQ	5	85	7.0	0.2
	Overall	10	86	8.0	n/a
'C'	LOQ	5	107	5.3	n/a
	10x LOQ	5	110	5.3	0.0
	100 x LOQ	5	109	5.1	0.0
	Overall	15	109	5.0	n/a

NOTE: n/a = not applicable

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