

LABORATORY SIMULATION STUDIES OF LEACHING OF THE PRIORITY PESTICIDES AND THEIR TRANSFORMATION PRODUCTS IN SOILS

M. Suleman^{1,2*} and B. J. Keely¹

¹Department of Chemistry, University of York, York, United Kingdom, YO10 5DD,

²Department of Agricultural Chemistry, The University of Agriculture, Peshawar, Pakistan, 25130,
Corresponding author e-mail; suleman@aup.edu.pk

ABSTRACT

Four priority pesticides (isoproturon, thifensulfuron methyl, glyphosate and propyzamide) and their main transformation products (TPs; monodesmethyl isoproturon, thifensulfuron acid, aminomethyl phosphonic acid and a ketone metabolite) were investigated for leaching behaviour from sandy clay loam soil contained in polyvinyl chloride pipes under laboratory conditions. Pesticides and their TPs were applied separately to different soil cores at the maximum recommended rates. The soils were irrigated and leachates were collected each week. Analysis of pesticides and TPs were performed via liquid chromatography mass spectrometry. It was found that isoproturon, monodesmethyl isoproturon, glyphosate, and aminomethyl phosphonic acid showed significant leaching behaviour, and were detected in leachates after a lag phase of 3-4 weeks. Propyzamide and thifensulfuron methyl were not detected in leachates nor were their TPs. Aminomethyl phosphonic acid showed a weak leaching tendency by comparison with glyphosate. It is evident that polarity as well as the ionic properties of the pesticides is important for leaching in clay loam soil. Being more polar, TPs should also be considered during risk assessment and monitoring studies of surface and ground waters.

Key words: Pesticides, transformation products, pesticide leaching, soil columns, ground water contamination,

Abbreviations: IPU, isoproturon; MDIPU, monodesmethyl isoproturon; DDIPU, didesmethyl isoproturon; TSM, thifensulfuron methyl; TSA, thifensulfuron acid; GLYP, glyphosate; AMPA, aminomethyl phosphonic acid; PROP, propyzamide; TPs, transformation products; LC-MS, liquid chromatography-mass spectrometry; LC-MSⁿ, liquid chromatography-tandem mass spectrometry; CID, collision induced dissociation; Da, daltons; SPE, solid phase extraction; NL, neutral loss.

INTRODUCTION

Pesticides are the most commonly detected organic pollutant in ground and surface water. Generally, pesticides with polar or moderately polar in nature can easily wash off from the plants leaves and run off from soil surface and hence leach down to ground water (Canle *et al.*, 2001). Pesticides and their transformation products (TPs) are frequently detected in aquifers worldwide (Kolpin *et al.*, 1996; Battaglin *et al.*, 2000; Landry *et al.*, 2005; Polati *et al.*, 2006; Hernández *et al.*, 2008). Generally, TPs are considered less toxic than the parent compounds (Nawab *et al.*, 2003), although in some cases they have been found to have greater toxicity (Osano *et al.*, 2002). The TPs are often more mobile and can be present in ground and surface water at higher levels than the parent compounds, thus, presenting a potential threat to the environment (Sinclair *et al.*, 2003; Andreu and Picò, 2004). The more mobile TPs can migrate more readily from the site of application and enter surface waters, ground waters and ultimately the marine environment (Broholm *et al.*, 2001). Thus, contamination by pesticides and TPs has been traced to rivers and reservoirs (Battaglin *et al.*, 2000). Some pesticide TPs have also been found in raw and treated drinking waters

where they have the capacity to affect human health (Coupe and Blomquist, 2004). As the toxicological evaluation of TPs is an emerging issue, their risk in source drinking water needs to be considered (Ferrer and Thurman, 2003; Richardson, 2004; Sinclair and Boxall, 2003).

Pesticides degradation starts from the soil surface and continue gradually in sub surface and lower sub surface of soil. Environmental conditions such as temperature, moisture and different agricultural practices such as irrigation and tillage practices can affect pesticide degradation and leaching (McLaughlin *et al.*, 1994). Different physiochemical properties of soil like organic matter, soil texture, structure and pH also affect the mobility of pesticide in different environmental compartment and finally contaminate groundwater (Cox *et al.*, 1998; Katagi, 2013). High organic matter content in soil decreases the mobility of pesticides and vice versa. Polar and non polar pesticide show different behaviour in soil. Mobility of polar and ionic pesticides is greatly affected by pH and clay content of the soil (Andreu and Pico, 2004). Different parameters such as distribution coefficient (K_d), soil-sorption coefficient (K_{oc}) and half-life (DT_{50}) of pesticides in soil plays a vital role in pesticide persistent and mobility. The leaching potential

of pesticides and their TPs in soils can be predicted based both on monitoring data obtained from field and laboratory-based studies, and on the physicochemical properties of the molecules (Sinclair and Boxall, 2003; Haith, 2010; Kern *et al.*, 2011). Environmental risk assessment and prioritisation of pesticides and their TPs highlights the candidates most likely to present hazards. Thus, the approach identifies the pesticides and TPs that require the most immediate attention. Various approaches including field- and laboratory-based methods have been used for the assessment of the leaching potential of agrochemicals. Laboratory-based soil column experiments are usually used for the assessment of the fate of pesticides in soil and to assess their leaching to ground water (Kenneth, 1990; Conrad *et al.*, 2006; Sinclair *et al.*, 2006).

The present study employed soil columns to examine the fate and leaching potential of the priority compounds that were used in North Yorkshire. Priority list of the pesticide along with their TPs was devised and top ranked compounds were selected for laboratory based column studies.

MATERIALS AND METHODS

Chemicals and reagents: Acetonitrile, methanol, water, acetic acid and dichloromethane were of HPLC grade (Fischer Scientific UK, Loughborough, UK), 9-fluorenylmethyl chloroformate (FMOC-Cl) was purchased from Sigma-Aldrich (UK). Standards of IPU, TSM, GLYP, AMPA, FMOC-AMPA, FMOC-GLYP and PROP (purity >99.0%) were purchased from Q_{max} laboratories (Thaxted, UK).

Soil sampling: A sandy clay loam soil collected from an uncultivated field (Heslington, York, UK), was used for the soil column study. The site had not been subject to pesticide treatment in the preceding 2 years. Soil was collected from two depths, 0-15 cm and 15-25 cm, at 9 different sample points throughout the field. Each of the respective surface and sub-surface soils was mixed thoroughly to obtain a homogenous surface and subsurface soil. Both surface and subsurface soils were air dried immediately after collection and sieved through a 2 mm screen. Physico-chemical properties of the soil (surface and subsurface soils were mixed together for this purpose) measured before the start of the experiment were: total organic carbon (1.28%), soil texture (sandy clay loam), pH (6.8) and bulk density (1.5g cm⁻³).

Preliminary leaching study: A preliminary study of the leaching of pesticides and their TPs was conducted to evaluate the soil columns for possible edge effects or water logging problems. For this purpose 200 µg TSM (10 × recommended application rate) dissolved in 1 mL of water and acetonitrile (50:50; v/v) was applied to the soil column. The columns (duplicate) were irrigated with

artificial rain (0.01 M CaCl₂ solution in deionised water (OECD, 1993)) each week for four weeks. The volume of the applied artificial rain (approx. 45 mL) corresponded to the average rainfall in York (UK) for the months September-October. None of the columns was found to have either edge effects or water logging problems. The rest of the experiments were conducted in a similar manner as detailed below.

Soil columns and experimental conditions: Polyvinyl chloride (PVC) drainage pipes (height 30 cm, diameter 6.4 cm) were used for the laboratory soil column studies. A sieve (screen size ≤ 2 mm) and cotton wool (≤ 1 mm thickness) was placed at the bottom of each column to prevent movement of soil particles into the receiving flask along with the leachate. Each column was filled with a homogenized soil mixture to a height of 26 cm. The depth profile from the sample site was re-constituted in the cores, with the surface soil (0-15cm) overlying the deeper soil (15-25 cm). In order to achieve uniform packing of the soil columns, each column was weighed (*c.* 1.3 kg column⁻¹) after packing and adjusted to the desired value by the addition or removal of the soil from the surface. A filter paper was placed at the top of each soil surface to prevent disturbance of the soil surface during irrigation of the column. The soil columns were taken to the field water capacity level by placing the soil columns in a water container overnight to allow them to become saturated via absorption and capillary movement. In order to remove trapped air, all columns were pre-wetted with 0.01 M CaCl₂. All of the experiments were conducted at *c.* 23 ± 2°C. The design of the study aimed to minimise the effects of the soil structure, which are highly variable in clay enriched soil. The utility of this approach enables a higher level of control than in systems that may more closely represent the natural variability (e.g. in field lysimeters or undisturbed soil columns).

Application of pesticides and their TPs: Analytical standards of the individual pesticides and the TPs MDIPU and AMPA, dissolved in water: acetonitrile (1:1 v/v), were applied separately to different columns in a single dose at the maximum recommended dose level. Thus, IPU and MDIPU were applied at 1.25 kg ha⁻¹, GLYP and AMPA at 1.080 kg ha⁻¹, and TSM and PROP at 120 g ha⁻¹ (2 × maximum recommended rate/ha due to a very low recommended field application rate) and 2.40 kg ha⁻¹, respectively. All columns, including control columns where no pesticides or TPs were applied, were run in duplicate.

Irrigation and leachate collection: The soil columns were irrigated weekly with CaCl₂ solution (0.01 M) as an artificial rain (Chopra *et al.*, 2010). The solution was applied as a single batch (assuming worst case scenario) during evening, and approx. 35-40 mL of leachate (~90-95% of applied artificial rain) was collected from each

column the following morning. Irrigation and sampling commenced 24 h before pesticide or TP application, at 24 h after application and then progressively every seven days from day 7 to day 49. On day 50 the soil columns were sliced and an inner section (2-2.5 cm² × 25 cm) was taken from the centre of each column. Both leachates and the inner cores were analysed for pesticides and their TPs.

Extraction of the pesticides and their TPs from leachates: Pesticides and their TPs were extracted from leachates by solid phase extraction (SPE) using Oasis HLB SPE cartridges (3 mL, 60 mg; Waters Corporation, Milford, USA). In the cases of GLYP and AMPA, the analytes were derivatised with FMOC-Cl prior to SPE (Hanke *et al.*, 2008). For IPU, MDIPU and TSM extraction, SPE cartridges were pre-conditioned with methanol and deionised water. Leachates were loaded onto the cartridges at a flow rate of 4 mL min⁻¹ and the cartridges dried under vacuum for 30 min. Elution of IPU and its TPs was carried out with acetonitrile, while acidified acetonitrile (2% acetic acid) was used to elute TSM. PROP was extracted by SPE using a Varian C₁₈ cartridge (6 mL, 120 mg; Darmstadt, Germany), with pre-conditioning, sample loading and drying steps as described earlier. The analytical elution of the Varian SPE cartridges was carried out using methanol/acetone (3:1 v/v). The details of the SPE procedures are given in **Table 1**.

Extraction of pesticides and their TPs from the soil: IPU and its TPs were extracted from the soil samples using the method developed by Mosleh *et al.* (2003). Briefly, a c. 5 g sample taken from the homogenised inner soil core was suspended in methanol (15 mL) and agitated on a mechanical shaker for 2 h prior to the supernatant being collected. Extraction was repeated twice with fresh methanol. After centrifugation of the pooled supernatants, the final volume was dried by vacuum centrifugation. Dried extracts were stored at 4°C prior to analysis.

GLYP and AMPA were extracted from the soil by the method of Landry *et al.* (2005). Approx. 50 g of the homogenised inner core was suspended in deionised water (100 mL), and shaken for 10 h. Following centrifugation, the supernatant was collected and derivatised with FMOC-Cl for further analysis (Hanke *et al.*, 2008). PROP and its TPs were extracted from the soil using the method of Exposito and Walker (1998). Briefly, 20 g of homogenised inner core soil was suspended in acetonitrile (25 mL) and shaken for 1 h before being allowed to settle. The supernatant was subsequently collected and dried in vacuo.

Instrumental analysis

Electrospray ionisation liquid chromatography mass spectrometry (ESI LC MS): TSM was analysed using a

Dionex RSLC 3000 system (Camberley, UK) equipped with a quaternary solvent delivery system and a diode array detector. Chromatographic separation was achieved using a Phenomenex Prodigy ODS3 column (250 × 4.6 mm; 5 µm; Macclesfield, UK) using a binary solvent system comprising 0.02 M ammonium acetate solution adjusted to pH 6.00 (A) and acetonitrile (B). The gradient program was: 10% B (0 min), 20% B (10 min), 40% B (25 min), 90% B (30 min), returning to the initial condition at 35 min. A flow rate of 0.7 mL min⁻¹ was used. The chromatograph was coupled to an electrospray ionisation (ESI) source installed on a Bruker HCT ultra ETD II ion trap mass spectrometer (Bruker Daltonics, Coventry, UK). The ESI conditions were optimized during infusion of a standard solution of TSM directly into the ESI source, with negative ionisation mode employed. Mass spectral operating parameters included: scan range *m/z* 50-1000, drying gas temperature 345°C, nebuliser pressure 44 psi and dry gas flow rate 8.00 L min⁻¹. Collision induced dissociation (CID) was achieved using a fragmentation amplitude of 0.45 V with isolation width of 1.5. Multistage tandem mass spectrometry was performed up to MS³ by automatic selection of the base peak ion following each stage of dissociation. Tandem mass spectra reported are those of diagnostic value.

ESI LCMS/MS of IPU and its TPs: An Xterra MS C₁₈ column (100 mm × 4.6 mm, 5 µm, Waters Corporation, Milford, USA) was used with a binary mobile phase system comprising 0.01 M ammonium acetate (A) and acetonitrile (B). The gradient program was: 43% B (0 min), 46% B (3 min) and 52% B (6 min), returning to the starting composition at 7 min. The flow rate was set to 0.8 mL min⁻¹ and the column was held at 30°C. Sample was dissolved in the mobile phase (57% A / 43% B) and 10 µL was injected. The ESI conditions were optimized during infusion of a standard solution of IPU and its TPs directly into the ESI source, with positive ionisation mode employed. Ionisation parameters included: scan range *m/z* 50-500, drying gas temperature 345°C, nebuliser pressure 35 psi and dry gas 8.00 L min⁻¹. CID was achieved using fragmentation amplitude of 0.80 V, isolation width of 2.00 and with smart Frag employed with a range of 30-200%. Multistage tandem mass spectrometry was performed up to MS² by automatic selection of the base peak ion and tandem mass spectra reported are those of diagnostic value.

ESI LC MS/MS of GLYP and AMPA: Chromatographic separation was achieved using Zorbax C₈ column (250 × 4.6 mm; 5 µm, Agilent, Berkshire, UK). The LC mobile phase gradient employed 10 mM ammonium acetate (A) and acetonitrile (B). The gradient program commenced with organic modifier: 10% B (0 min), increasing gradually to 90% B (15 min) and reverting to the initial composition at 20 min. The flow rate was 0.7 mL min⁻¹ and an injection volume of 10 µL

was used. Positive mode ESI was optimised by direct infusion of standard solutions of FMOc-GLYP and FMOc-AMPA. The MS/MS analysis of both FMOc-GLYP and FMOc-AMPA yields diagnostic ions that permit their identification (Ibáñez *et al.*, 2005). The ESI acquisition parameters were: scan range m/z 50-1000, maximum acquisition time 200 ms, smart target 200000, drying gas temperature 320°C, drying gas flow rate 9.0 L min⁻¹, nebuliser pressure 40.0 psi, target mass 392 and fragmentation amplitude 0.8 V with isolation width 2.

ESI LC MS/MS of PROP and its TP: A reversed phase LC method was developed for separation of PROP and its TPs, employing an Xterra MS C₁₈ column (100 × 4.6 mm; 5 μm, Waters, Hertfordshire, UK) and a mobile phase comprising 0.01 M ammonium acetate (A) and methanol (B). The gradient programme started with 50% A and 50% B (0 min) changing to 100% B (15 min) before returning to the starting composition at 20 min. The injection volume was 10 μL. Negative mode ESI conditions were optimised for PROP by infusion of standard solution of PROP. The ESI acquisition parameters were: scan range m/z 50-500, maximum acquisition time 200 ms, smart target 200000, drying gas temperature 365°C, drying gas flow rate 9.0 L min⁻¹, nebuliser pressure 40.0 psi, target mass 254 and fragmentation amplitude 1.0 V with smart fragmentation range of 100 to 200% and isolation width 2.

RESULTS AND DISCUSSION

An analysis of pesticide usage data for North Yorkshire during the years 2006-07 and 2007-08 enabled the risk assessment and prioritisation approach reported by Sinclair *et al.* (2006) to be applied over a two year period to identify the top priority pesticide TPs on the basis of several key factors including the amount of the parent pesticide used, the propensity for formation of TPs and their mobility and persistence in the environment. Physicochemical properties of TPs, their metabolism and pathways were identified from the literature and the pesticide safety directorate (PSD, UK). Generally, TPs were selected that are usually formed by physical, biological or biochemical process and are reported in the literature. Thus, the TPs were ranked according to their exposure values from the risk assessment process. As a variety of data sources have been used during risk assessment and prioritisation, the quality and accuracy can vary. To account for this, the TPs were divided into different categories depending on the nature and / or source of the data available for use in the risk assessment scheme. TPs for which all the values used during risk assessment were based on laboratory / experimental observations assigned as A class and were selected for present study. The priority components thus identified and their precursors are monodesmethyl isoproturon

(MDIPU) from isoproturon (IPU), aminomethyl phosphonic acid (AMPA) from glyphosate (GLYP), thifensulfuron acid (TSA) from thifensulfuron methyl (TSM) and N-(1,1-dimethylacetyl)-3,5-dichlorobenzamide, also known as RH24580, from propyzamide (PROP) (Fig. 1).

Methods development for the analysis of pesticides and their TPs

TSM and its TPs: Due to the lack of a reliable and rapid LC-MS method for the simultaneously analysis of TSM and its TPs, a reversed phase ESI LC-tandem mass spectrometric method was developed for the analysis of TSM. Being a weak acid, elution of TSM was accomplished with the aid of an acidic buffer. Use of strong acidic or alkaline solutions can cause breakdown of sulfonylureas to their TPs (Cambon and Bastide, 1996), hence the organic salt, ammonium acetate, was added to the mobile phase. Thus, a C₁₈ reversed phase column was used with aqueous ammonium acetate buffer (0.01 M) containing acetonitrile as an organic modifier. Initial studies showed that buffering the solution at pH 7 led to peak tailing, most likely due to interaction with silanol sites. Increasing the ionic strength of the buffer to 0.02 M slightly improve the peak shape. Further improvement in peak shape and a reduction in retention time were achieved on reducing the pH of the buffer to 6.0. Accordingly, subsequent analysis was performed using 0.02 M ammonium acetate at pH 6.0 (**Fig. 1**). During tandem mass spectrometry (MS/MS) of TSM, three diagnostic product ions were generated (**Fig. 2**). The MS/MS of the deprotonated molecule ([M-H]⁻) at m/z 386 produces ions at m/z 220 (corresponding to the 2-ester-3-sulfonamide anion), m/z 188 (thiophene sulfonimide anion) and m/z 139 (triazine amine anion). Further dissociation of the MS² ion at m/z 220 produced an intense ion at m/z 188 in MS³ via loss of methanol (32 Da; **Fig. 3**).

IPU and its TPs: The ESI MS/MS spectrum of protonated IPU (m/z 207) shows product ions at m/z 165 and m/z 72 resulting from neutral losses of propene (-42 Da) and 4-isopropylaniline (-135 Da), respectively (Belmonte *et al.*, 2005). Similarly, MS/MS of protonated MDIPU (m/z 193) yields intense product ions at m/z 151 ([M+H-42]⁺) and 136, which correspond to neutral losses arising from the same two bond cleavages (Amorisco *et al.*, 2005). The MS/MS spectrum of protonated DDIPU (m/z 179) produces major product ions at m/z 162 ([M+H-NH₃]⁺) and m/z 137 ([M+H-42]⁺) (**Fig. 4 and 5**).

GLYP and AMPA: Positive mode ESI LC-MS of FMOc-AMPA gave a protonated molecule at m/z 334 from which a product ion at m/z 179 (fluorenyl methylum) was formed via neutral loss of AMPA (-111 Da) during CID (**Fig. 6**). Another dissociation pathway of the protonated molecule yielded an ion at m/z 156 due to

loss of methylene fluorene, ultimately producing an ion at m/z 112 by loss of CO_2 to form an AMPA cation (Fig. 7). Similarly, FMOC-GLYP gave a protonated molecule at m/z 392 (Fig. 6) which also followed two distinct dissociation pathways during CID. A product ion at m/z 214 in MS/MS, formed by neutral loss of methylene fluorene (-178 Da), dissociated further by loss of CO_2 to give an ion at m/z 170, corresponding to protonated GLYP, in MS^3 . The second pathway yielded an ion at m/z 179 (fluorenyl methylum) via neutral loss of GLYP (-169 Da) and CO_2 (-44 Da) (Fig. 8).

PROP and its TP: The negative mode ESI LC-MS base peak chromatogram for the analysis of PROP shows a deprotonated molecule at m/z 255 (Fig. 9) which, on CID, produced a product ion at m/z 228 by neutral loss of methanol. Further dissociation of this product ion yielded ions at m/z 213, formed via neutral loss of CH_3 (-15 Da), at m/z 187, formed via loss of propene, and at m/z 145, corresponding to the dichlorobenzene ion in MS^3 (Fig. 10). Similarly a ketone TP of PROP (RH24580) yields a deprotonated molecule at 272 that on CID yields three ions suitable for its assignment: m/z 213, m/z 145 and m/z 126.

Leaching of pesticides and their TPs

TSM-treated soil columns: Sulfonylurea herbicides are widely regarded as being moderately to highly mobile in soil and hence as posing a potential risk to ground water. In common with other sulfonylurea herbicides, the field recommended application rate of TSM is very low (15-60 g ha^{-1}) as it is a highly potent herbicide. TSM was observed in the leachates of the preliminary study at 9.5 % of the amount applied (equivalent to 600 g ha^{-1} , $\times 10$ the recommended dose level). When applied at a rate of 120 g ha^{-1} ($\times 2$ recommended dose level), neither TSM nor its TPs were detected in any of the leachates. Extraction from the soil revealed levels of TSM of 0.030 $\mu\text{g g}^{-1}$ (20.5 %) of soil at the end of the preliminary experiment and 0.007 $\mu\text{g g}^{-1}$ (18 %) of soil at the end of the main experiment. TSM is highly soluble in water and its presence in the effluents of the preliminary soil columns presumably resulted due to the high loading causing oversaturation of the column. The mobility of TSM is influenced in part by soil pH (Beyer, 1988) and partly by adsorption onto organic matter by formation of hydrogen bonds, inorganic cation interactions or by binding via hydrophobic bonds (Lagana *et al.*, 2000).

Chemical and microbial degradation pathways are important in the transformation processes of sulfonylurea herbicides in soils (Brown *et al.*, 1990). The degradation rate of TSM in soil is much faster than those of the other herbicides of the same group and microbial degradation is the more common pathway (Stevens and Duxbury, 1992; Cambon *et al.*, 1998). The biological de-esterification of TSM in soil can be effected by several

soil micro flora, these having been shown to be capable of de-esterification of TSM in pure culture (Brown *et al.*, 1997). Hydrolysis of TSM, a prominent reaction in its decomposition pathway, depends on the pH of the solution, having a half-life of 1.5 days at pH 5 and around 10 days at pH 7 at 25°C (Brown *et al.*, 1990). The hydrolysis of TSM under acidic conditions involves in the cleavage of the sulfonylurea bridge and demethylation of the methoxy group. Under alkaline conditions hydrolysis leads to the formation of thifensulfuron acid (TSA) by saponification of the methoxy ester substituent (Cambon and Bastide, 1996). Generally TSA, which has no herbicidal activity, has been reported as an initial degradation product in soils (Brown *et al.*, 1997; Cambon *et al.*, 1998). Soil moisture also plays a role in the degradation rate of TSM, which decreases proportionately with an increasing water/soil ratio (Cambon *et al.*, 1998). Thus, the rapid disappearance of TSM from the soil during the present study might be as a result of the drying periods between each irrigation interval. No TSA was detected during the study, suggesting comparatively less stability of the TSA compared with its parent TSM in the prevailing circumstances. It is important to note that soil does strongly retain TSM up to its binding capacity, the retention making it unavailable for microbial or chemical degradation.

The results from the preliminary study and the main study indicate that the application rate can play an important role in the leaching behaviour of TSM and hence, by implication, for other sulfonylurea and related pesticides. Thus, studies at higher application rates ($\times 10$ -100) should be carried out to investigate the leaching potential of TSM at these higher application rates and to monitor the fate of elevated TSM concentration due to either repeated application, illegal use or accidental spillages. The intensity of rainfall might also play an important role in the leaching of TSM in soils with very low organic matter contents. After a fresh application of TSM, heavy rainfall might result in a substantial leaching or surface runoff of the pesticide to either surface or ground water. Accordingly, TSM and its TPs should be monitored during and after such conditions.

IPU- and MDIPU-treated soil columns: The phenylurea herbicide IPU was applied to soil columns at the recommended field application rate. The leachate data show that neither IPU nor MDIPU were present in the leachates collected during the first three weeks (Fig. 11). During week 4 to week 6 IPU and MDIPU were present in the leachates together with DDIPU, a further TP which can undergo deamination to produce 4-isopropylaniline (4-IA). An alternative pathway involves the hydroxylation of the isopropyl group of MDIPU and ultimately leads to the 4-IA or its derivatives (Mudd *et al.*, 1983; Juhler *et al.*, 2001). No 4-IA or its derivatives

were detected during the present study. The overall recoveries from the IPU-treated soil column eluates were 3.1% (12.6 µg) for the IPU, 2.5% (10.10 µg) for MDIPU and 1.5% (5.6 µg) for DDIPU. The recoveries from the eluates indicate the leaching potential of IPU and its main TPs, MDIPU and DDIPU through the soil columns. None of the above compounds were found in the week 7 samples. Both IPU and its TP DDIPU were also extracted from the soil at the end of the experiments, the molar abundances for total IPU and TPs recovered representing 4.9% (0.015 µg g⁻¹) of the total applied IPU. The calculations were performed by balancing the amount of IPU and DDIPU recovered from the soil core to the total amount of IPU applied to the soil column.

Thus, leachate recoveries of IPU and its TPs were higher than the recovery of IPU from the soil at the end of the experiments. The mineralisation of IPU can also be rapid in nitrogen deficient soils where microorganisms use IPU and its TPs as a sole source of C and/or N. The abundance level of IPU presumably reflects its binding capacity within the soil. Notably, however, no MDIPU was detected. The absence of MDIPU and presence of DDIPU suggests either rapid demethylation of MDIPU to DDIPU in the soil or a greater retention of MDIPU by the soil. Given the elution profiles of IPU and its TPs from the soil columns the former explanation appears much more likely.

Soil columns treated with MDIPU showed its presence together with one of its main TPs, DDIPU, in the leachates collected from weeks 3 to 6 (**Fig. 12**). The leachate recoveries of MDIPU and DDIPU were 3.1% (13 µg) and 6.1% (24.1 µg) of the applied amount of MDIPU, respectively. The highest concentration of DDIPU was found during week 3 after which the level declined sharply to the end of week 6. A similar trend was observed for MDIPU, though no MDIPU was found in the week 6 samples. Similarly, MDIPU and its TPs extracted from the soil represented 3.2% (0.01 µg g⁻¹) of the MDIPU applied. Thus, the leachate contributed a higher value of MDIPU and its TP DDIPU (9.2%) than that of the amount retained by the soil at the end of the experiments (**Fig. 13**).

The data for both IPU- and MDIPU-treated soil columns reveal the presence of the parent compounds and their respective TPs in the leachates. High levels of MDIPU and DDIPU were formed during the study. Relatively slow migration of IPU restricts its leaching through the columns during the first three weeks. The higher amounts of IPU and its main TPs MDIPU and DDIPU in the leachate during week four suggest the elution of IPU from the soil columns and that its rapid degradation to MDIPU and DDIPU had occurred in the soil columns. The degradation of IPU via successive demethylation (removal of N-methyl group) to produce MDIPU and then DDIPU, has been suggested to influence degradation rate: demethylation of IPU to

produce MDIPU has been proposed as the rate limiting step in the complete degradation of IPU (Sørensen and Aamand, 2001). Once MDIPU is formed, it undergoes rapid transformation to produce DDIPU. IPU and its TPs have been detected in surface and ground waters in concentrations exceeding the EU limit of 0.1 µg L⁻¹ (Jiang *et al.*, 2011). Due to the inference that it leaches to surface and ground waters (Spliid, 2002), IPU was included in the phase-out list of the products and its further use was banned in the UK since June 2009.

The pesticide usage data for both 2006-07 and 2007-08 showed that IPU was one of the most extensively used pesticides in North Yorkshire (United Kingdom) during those particular time periods; hence its placement among the top priority compounds in the risk assessment. The risk assessment and prioritisation, and finally soil column experiments, confirmed that IPU and its main TPs can pose a risk to ground water, and that they should be monitored routinely in both environmental surface and ground waters. Although IPU has been included in the phase out products, its monitoring in the environmental samples could remain helpful in identifying cases of illegal use as well as in countries where its use is permitted.

GLYP and AMPA-treated soil columns: GLYP was applied to the soil columns at the maximum recommended application rate. GLYP recoveries from the leachates of the GLYP-treated soil columns represented 1.4% of the total amount of GLYP applied (**Fig. 14**). Similarly a small fraction of GLYP transformed into AMPA (1.1%) was also detected in the leachates (**Fig. 15**). No GLYP was detected during initially collected leachates, it being detected only after week 3 to 5. AMPA was also recovered from the leachates between week 4 and 6. No GLYP was found in the leachates collected after week 6. Soil analysis of GLYP-treated soil column revealed that 18.80% (0.05 µg g⁻¹ of the soil) of the applied GLYP was present in the soil.

The summed AMPA recovery of leachates collected from the AMPA-treated columns was 0.9% (3.5 µg) of the applied amount. AMPA was detected in leachates collected after week 4 and 5 leachates. AMPA from GLYP-treated soil was found even in the leachates collected after week 6 though no AMPA was detected from AMPA-treated soil columns after week 6. AMPA recovered from the soil was 11.2% (0.03 µg g⁻¹ of the soil) of that applied. The lower values for AMPA in leachates (0.9%; 3.15 µg) compared with the proportion of GLYP in the leachate from GLYP treated columns indicates the restricted mobility and lower persistence of AMPA in the soil as compared to its parent GLYP (**Fig. 16**).

The soil columns leaching data for GLYP and AMPA shows that GLYP was detected in the leachates in

higher levels than AMPA despite AMPA being chemically more polar than its parent, GLYP. The leachate recoveries for GLYP were higher from the GLYP treated soil columns, suggesting comparatively high mobility of GLYP. However, GLYP is reversibly sorbed onto soil particles, resulting in slow dissipation of GLYP from soils. On release, GLYP undergoes microbial degradation to produce AMPA as the main TP (Kenneth, 1990; Franz *et al.*, 1997). The relatively high soil recoveries for both GLYP and AMPA explain their retention and sorption to soil particles and hence their slow dissipation through columns. The high amount of GLYP in the soil reflects the persistence of this pesticide (Franz *et al.*, 1997). The amount of AMPA was, however, very low and this could possibly be due either to strong sorption to soil particles or rapid mineralisation. GLYP and AMPA are considered to exhibit low mobility in soils due to their zwitterion nature and supposed strong sorption to the soil matrix (Sprankle *et al.*, 1975).

The soil used in the experiment was a sandy clay loam texture that restricts GLYP leaching, probably due to strong sorption of GLYP to clay particles. The results for the GLYP-treated columns also revealed a slow degradation of GLYP. The delayed elution of AMPA from both the GLYP- and AMPA-treated soil columns showed its stronger affinity to soil particles than GLYP. GLYP and AMPA have been found to be more mobile in soil that has a sandy loam texture even with high organic carbon content (Kenneth, 1990), hence, the organic matter content has little role in GLYP mobility in soils (Jacobsen *et al.*, 2008). Soil pH plays an important role in GLYP sorption to organic matter where stronger sorption being found at neutral pH than at acidic pH (Mamy *et al.*, 2005; Albers *et al.*, 2009). Soil type and

moisture content, however, can influence GLYP dissipation and mineralisation (Grundmann *et al.*, 2008). Together these two binding mechanisms account for the low mobility of the GLYP and AMPA observed during the present study.

PROP-treated soil columns: Being a relatively high application rate PROP was expected to be present in the leachates, however, PROP was not detected in any of the leachates. The low mobility and high persistence of the PROP was reflected from the soil samples of the PROP-treated columns. A known PROP TP, the ketone metabolite RH24580, was also detected in the soil treated with PROP. According to molar mass balance, about 18.6% ($0.11 \mu\text{g g}^{-1}$) of PROP and its TP were recovered from the PROP-treated soil column. The amount of RH24580 recovered from the soil contributed 5.1% ($0.030 \mu\text{g g}^{-1}$) and PROP 13.5% ($0.08 \mu\text{g g}^{-1}$) to the total recoveries (18.6%). The restricted mobility of PROP in the soil may be related to its poor solubility in water. The mobility of PROP and RH24580 are highly dependent on soil type. The organic matter and the high clay content of the loam soil might be responsible for the low mobilities of PROP and its TPs. The presence of both components in the soil at a relatively high amount revealed their slow mobility and degradation and strong sorption to the soil particles. The degradation of PROP varies under different environmental conditions. Temperature and dry-wet cycles play an important role by enhancing the rate of microbial degradation, especially in topsoil (Exposito and Walker, 1998), as microbial communities adapt to the PROP application after a lag phase of 5 weeks (Walker, 1992).

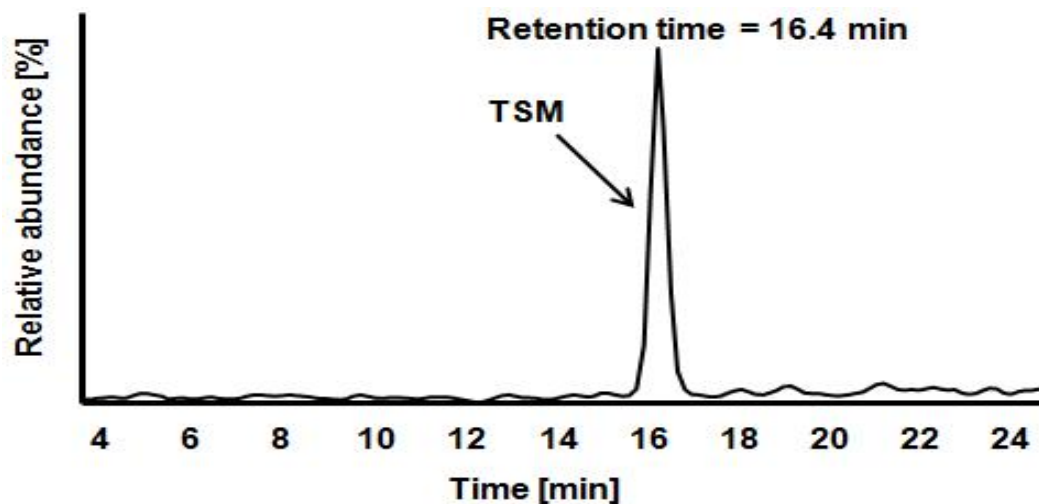


Fig. 1. LC-MS base peak chromatogram (-ESI) of a TSM standard.

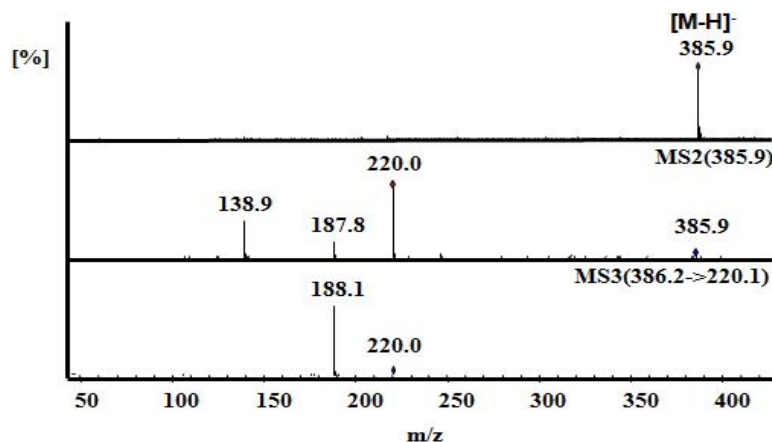


Fig. 2. Mass (-ESI) and multistage tandem mass spectra of TSM.

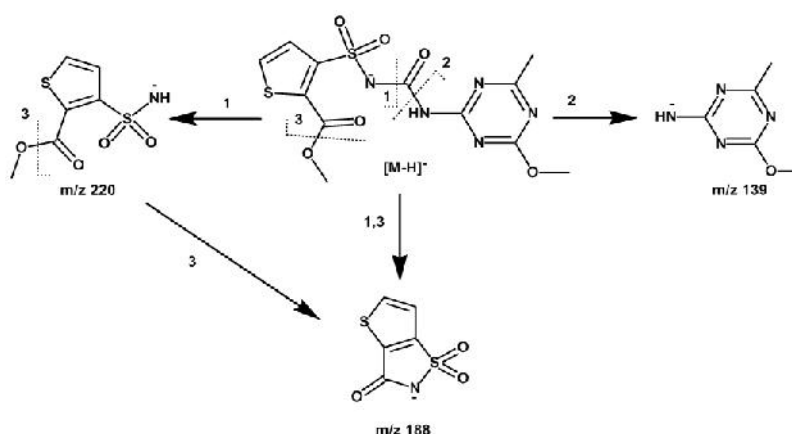


Fig. 3. Collision induced dissociation pathways for deprotonated TSM.

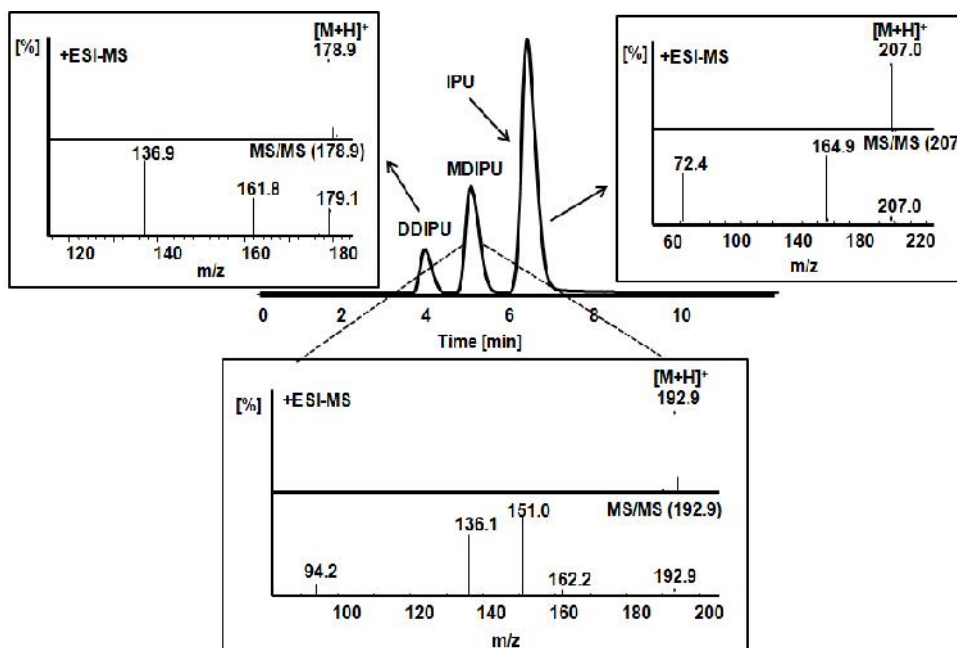


Fig. 4. LC-MS base peak chromatogram of a solution containing IPU, MDIPU and DDIPU, and associated mass (+ESI) and tandem mass spectra.

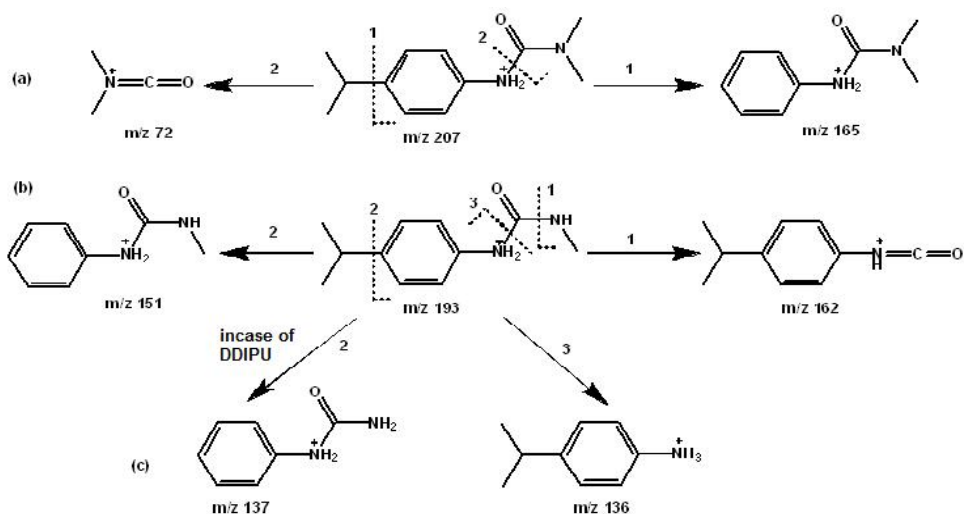


Fig. 5. Collision induced dissociation pathways for the protonated molecules of: a) IPU; b) MDIPU; c) DDIPU

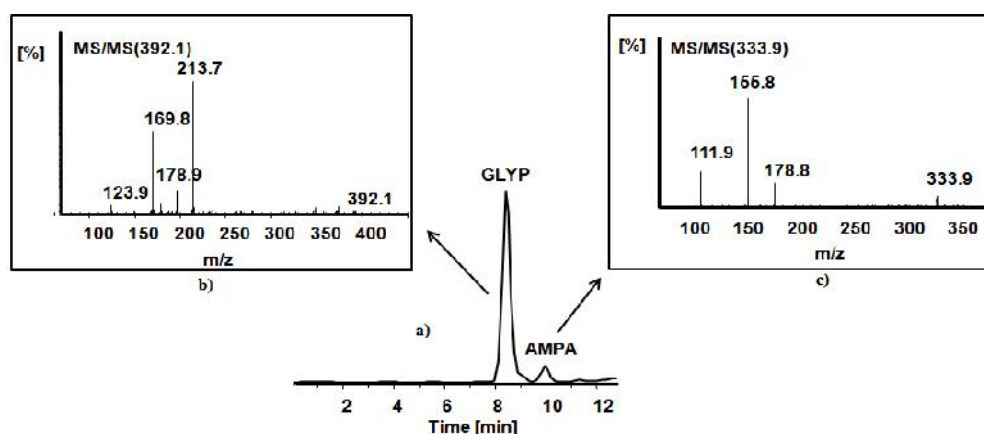


Fig. 6. a) LC-MS base peak chromatogram (+ESI) of FMOc-GLYP and FMOc-AMPA; b) MS/MS spectrum of FMOc-GLYP; c) MS/MS spectrum of FMOc-AMPA

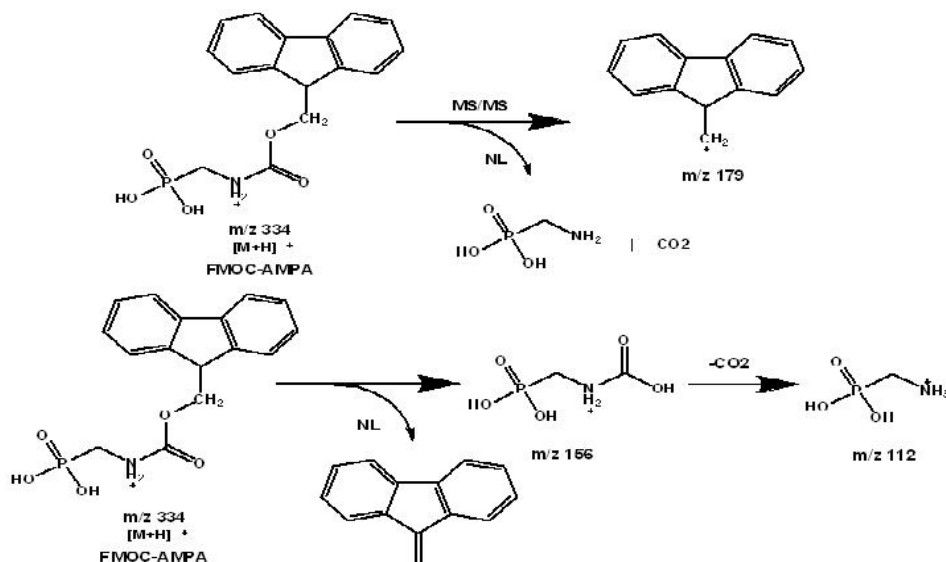


Fig. 7. Collision induced dissociation pathways of protonated FMOc-AMPA. The product ions m/z 179, m/z 156 and m/z 112 originate from the precursor ion at m/z 334.

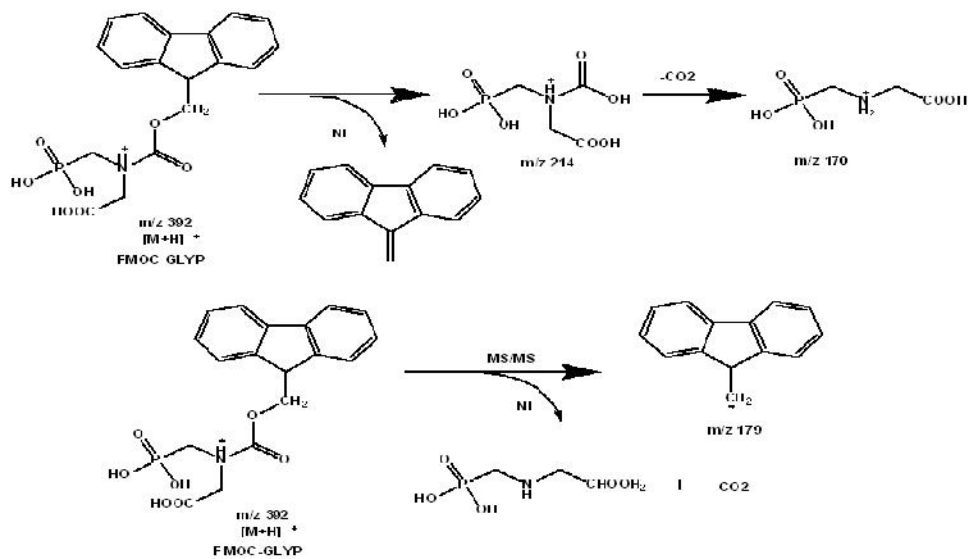


Fig. 8; Collision induced dissociation pathways of protonated FMOc-GLYP. The product ions m/z 214, m/z 170 and m/z 179 originate from the precursor ion at m/z 392.

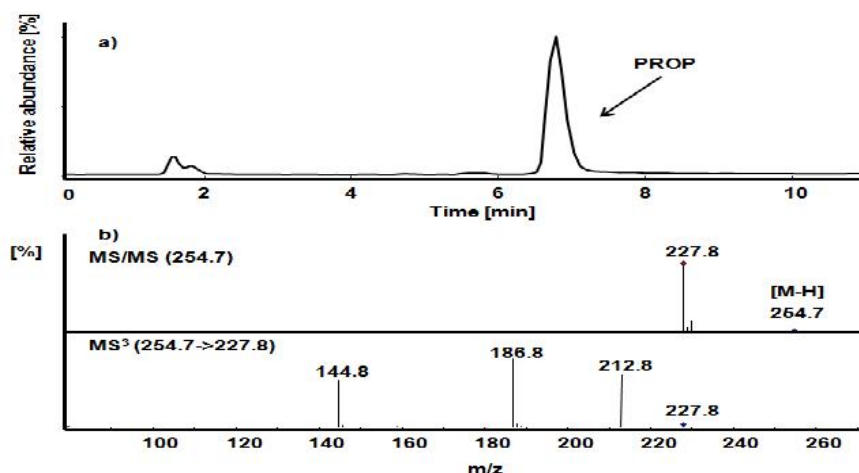


Fig. 9. a) LC-MS base peak chromatogram (-ESI) of PROP; b) multistage tandem mass spectra of PROP.

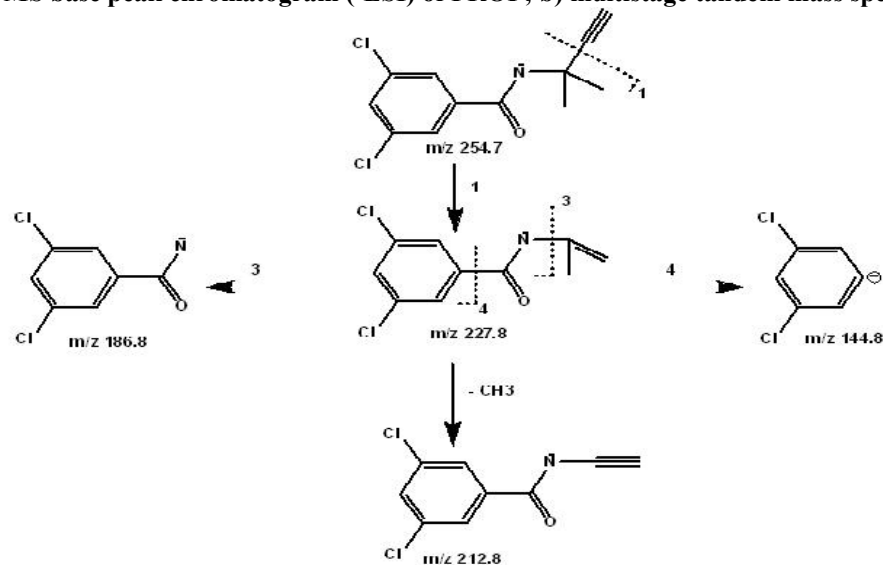


Fig. 10. Collision induced dissociation pathways for deprotonated PROP.

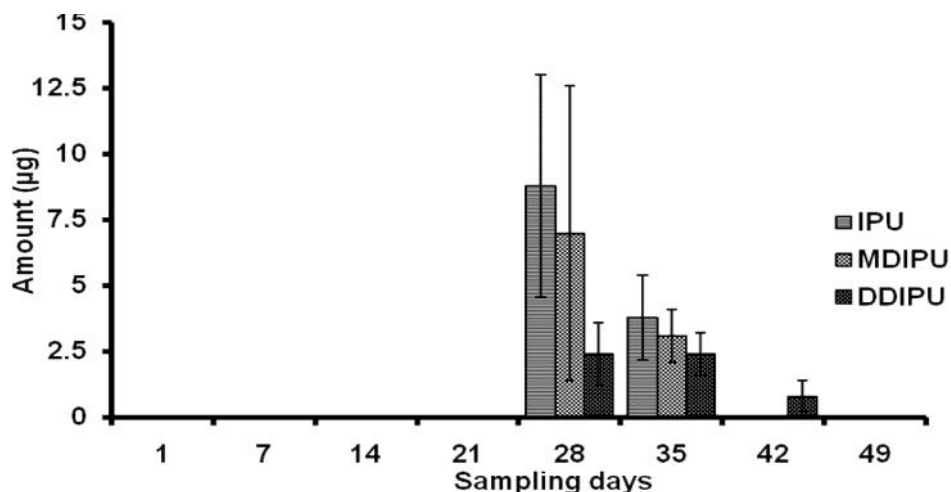


Fig. 11. Leaching profile of IPU and its TPs in IPU-treated soil columns. Error bars indicate the range of highest and lowest data points (n=2) and show the variability within the replicates

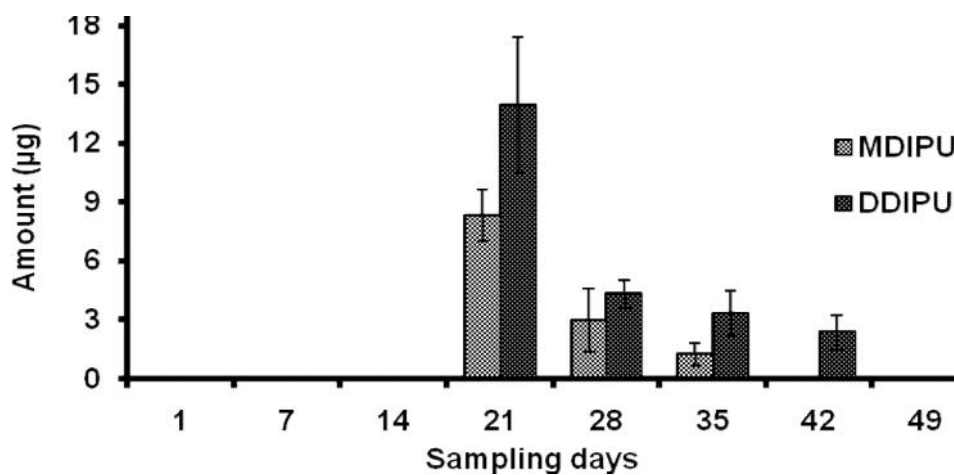


Fig. 12. Leaching profiles of MDIPU and its TPs in MDIPU-treated soil columns. Error bars indicate the range of highest and lowest data points (n=2) and show the variability within the replicates.

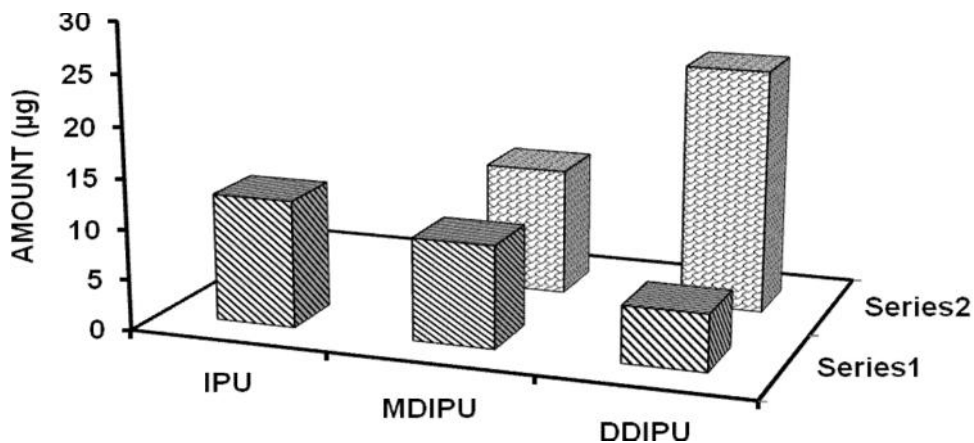


Fig. 13. Comparison of the recoveries of pesticides and their TPs from the IPU- (series-1) and MDIPU-treated (series-2) soil column leachates. The results show that similar recoveries were observed for MDIPU from the both IPU- and MDIPU-treated soil columns. Higher recoveries of DDIPU were observed for MDIPU-treated soil columns.

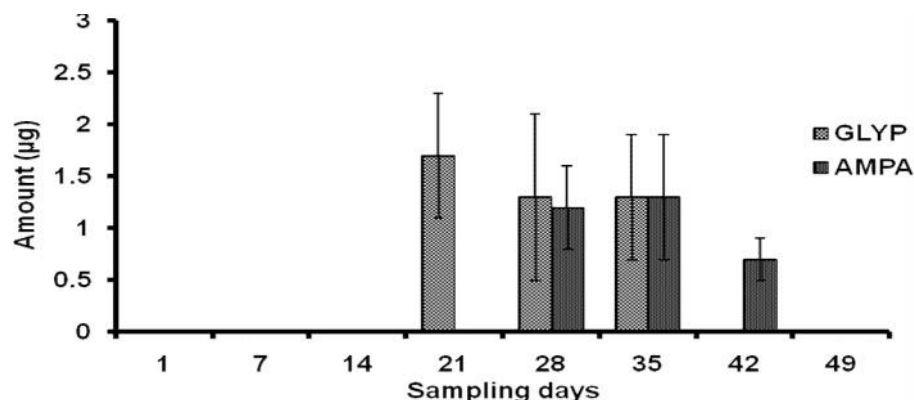


Fig. 14. Leaching profiles of GLYP and AMPA from the GLYP-treated soil columns. Error bars indicate the range of highest and lowest data points (n=2) and show the variability within the replicates.

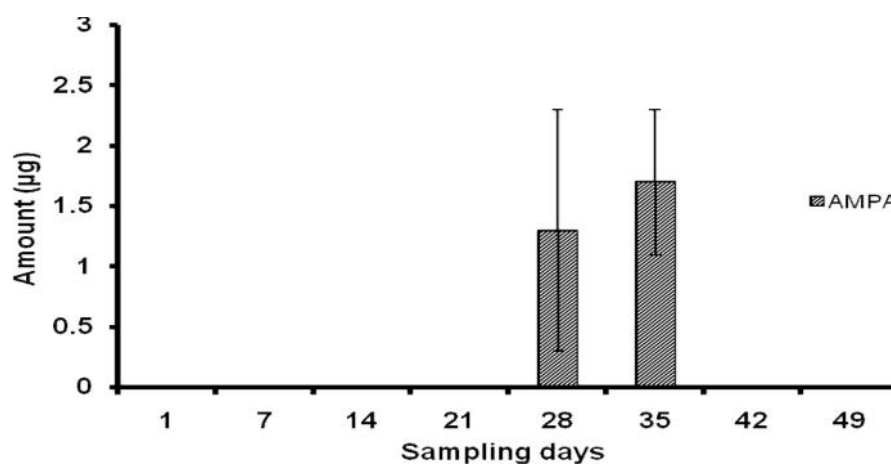


Fig. 15. Leaching profile of AMPA from the AMPA-treated soil columns. Error bars indicate the range of highest and lowest data points (n=2) and show the variability within the replicates.

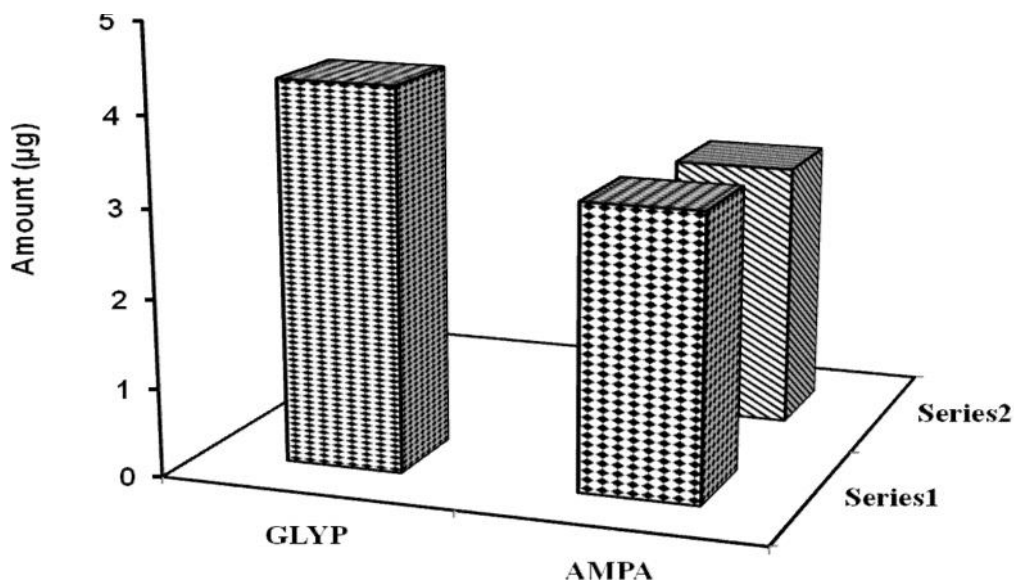


Fig. 16. Comparison of the recoveries of pesticides and their TPs from the GLYP- (series-1) and AMPA-treated (series-2) soil column leachates. The results show that similar recoveries were observed for AMPA from the both GLYP- and AMPA-treated soil columns. Higher recoveries of GLYP were observed for GLYP-treated soil columns.

Table 1. Details of the SPE methods used for the extraction of the pesticides and their TPs from the leachates.

Pesticide / TPs	Cartridge used	Pre-conditioning	Sample loading	Drying	Elution
TSM and its TPs	Oasis HLB	5 mL each of methanol and deionised water	4 mL min ⁻¹	30 min under vacuum	5 mL acidified acetonitrile (2 % acetic acid)
IPU, MDIPU and DDIPU	Oasis HLB	5 mL each of methanol and deionised water	4 mL min ⁻¹	30 min under vacuum	5 mL acetonitrile
GLYP and AMPA	Oasis HLB	5 mL each of methanol and deionised water (0.1 % formic acid)	4 mL min ⁻¹	30 min under vacuum	9 mL methanol without vacuum
PROP and its TPs	Varian C ₁₈	5 mL each of methanol and deionised water	4 mL min ⁻¹	30 min under vacuum	5 mL methanol/ acetone (3:1; V/V)

TSM, thifensulfuron methyl; TPs, transformation products; IPU, isoproturon; MDIPU, monodesmethyl isoproturon; DDIPU, didesmethyl isoproturon; GLYP, glyphosate; AMPA, aminomethyl phosphonic acid; PROP, propyzamide.

Conclusions: The leaching potential and degradation of four selected pesticides and their TPs. GLYP, IPU and their TPs have been revealed through their detection in the leachates. IPU and MDIPU were present at high concentrations in leachates compared to the other pesticides and their TPs. The highest amounts of IPU and MDIPU were detected in the leachates, indicating that IPU and MDIPU can pose a potential risk to ground water. The higher value of MDIPU than its parent compound in the soil column leachate also revealed that MDIPU is potentially more mobile than IPU. Although GLYP and AMPA were present in the leachates, their low levels categorises them as potentially low mobility compounds in this type of the soil. Contrary to IPU, GLYP is relatively more mobile than its TP AMPA and can potentially pollute ground water. Similarly, PROP and its TPs were not detected in the soil column leachates, however, the soil extract showed the persistent behaviour of the PROP and its TP RH24580. It can be concluded that pesticide TPs should also be included while doing risk assessment and a priority list should be devised each year for routine monitoring of high priority compounds in drinking, surface and ground waters.

Acknowledgment: We are grateful to, Alistair Boxall (FERA, Sand Hutton, UK) and Chris Knappy (University of York, UK) for their useful inputs and ideas. We thank the Higher Education Commission of Pakistan for their support and financial assistance. We would like to acknowledge the facilities and services provided by the Centre of Excellence of Mass Spectrometry at York, United Kingdom.

REFERENCES

- Amorisco, A. I. Losito, and F.P., P.G. Zambonin (2005). Photolytic degradation of the herbicide isoproturon: characterisation of by-products by liquid chromatography with electrospray ionisation tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 19, 1507-1516.
- Albers, C.N., G.T. Banta, P.E. Hansen, and O.S. Jacobsen (2009). The influence of organic matter on sorption and fate of glyphosate in soil comparing different soils and humic substances. *Environ. Pollut.* 157, 2865-2870.
- Andreu, V., and Y. Picò (2004). Determination of pesticides and their degradation products in soil: critical review and comparison of methods. *Tren. Anal. Chem.* 23, 772-789.
- Battaglin, W.A., E.T. Furlong, M.R. Burkhardt, and C. J. Peter (2000). Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the midwestern united states, 1998. *The Sci. Total Environ.* 248, 123-133.
- Belmonte, V., A. Garrido Frenich, and J.L.M. Vidal, (2005). Monitoring of pesticides in agricultural water and soil samples from Andalusia by liquid chromatography coupled to mass spectrometry. *Anal. Chimica Acta* 538, 117-127.
- Beyer, E.M., M.J. Duffy, J.V. Hay, and D. D. Schlueter (1988). In: P. C. Kearney, D.K. (Ed.), *Herbicides Chemistry, Degradation and Mode of Action*. Marcel Dekker., New York, pp. 117-189.
- Broholm, M., K. Ruge, N. Tuxen, A. Hojberg, H. Mosbaek, and P. Bjerg (2001). Fate of herbicides in a shallow aerobic aquifer: A continuous field injection experiment (vejen, denmark). *Water Resources Research* 37, 3163-3176.
- Brown, H.M., M.M. Joshi, A.T. Van, T.H. Carski, J.J. Dulka, M.C. Patrick, R.W. Reiser, R.S. Livingston, and J. Doughty (1997). Degradation of thifensulfuron methyl in soil: Role of microbial carboxyesterase activity. *J. Agric. Food Chem.* 45, 955-961.

- Brown, H.M., V.A. Wittenbach, R.D. Forney, and S. D. Strachan(1990). Basis for soybean tolerance to thifensulfuron methyl. *Pesti. Biochem. Physiol.* 37, 303-313.
- Cambon, J.-P., J. Bastide, and D. Vega (1998). Mechanism of Thifensulfuron-methyl Transformation in Soil. *J. Agric. Food Chem.* 46, 1210-1216.
- Cambon, J.P., and J. Bastide (1996). Hydrolysis kinetics of thifensulfuron methyl in aqueous buffer solutions. *J. Agric. Food Chem.* 44, 333-337.
- Canle, M., S. Rodriguez, L. F. R. Vazquez, J. A. Santaballa and S. Steenken (2001). First stages of photodegradation of the urea herbicides fenuron, monuron and diuron. *J. Mol. Struct.* 565. 133-139.
- Chopra, I., B. Kumari, and S. K. Sharma (2010). Evaluation of leaching behavior of pendimethalin in sandy loam soil. *Environ. Monit. Assess.* 160, 123-126.
- Conrad, A., O. Dedourge, R. Cherrier, M. Couderchet, and S. Biagianti (2006). Leaching of terbumeton and terbumeton-desethyl from mini-columns packed with soil aggregates in laboratory conditions. *Chemosphere* 65, 1600-1609.
- Coupe, R.H., and J. D. Blomquist (2004). Water-soluble pesticides in finished water of community water supplies. *J. AWWA* 96, 56-68.
- Cox, L., W. C. Koskinen, R. Celis, M. C. Hermosin, J. Cornejo and P. Y. Yen (1998). Sorption of Imidacloprid on Soil Clay Mineral and Organic Components. *Soil Sci. Soc. Am. J.* 62(4): 911-915
- Exposito, M. J., and A. Walker(1998). Degradation of isoproturon, propyzamide and alachlor in soil with constant and variable incubation conditions. *Weed Research* 38, 309-318.
- Ferrer, I., and E. M. Thurman (2003). Liquid chromatography/ time of flight-mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants. *Trends Anal. Chem.* 22, 750-756.
- Franz, J.E., M. K. Mao and J.A. Sikoerski (1997). Glyphosate a unique global herbicide. *A C S Monograph* 189, 653 p
- Grundmann, S., Dorfler, U., Ruth, B., Loos, C., Wagner, T., Karl, H., Munch J.C., and R. Schroll (2008). Mineralization and Transfer Processes of ¹⁴C-labeled Pesticides in Outdoor Lysimeters. *Water Air Soil Pollut.: Focus* 8, 177-185.
- Haith, D.A. (2010). Ecological Risk Assessment of Pesticide Runoff from Grass Surfaces. *Environ. Sci. Technol.* 44, 6496-6502.
- Hanke, I., H. Singer, and J. Hollender(2008). Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. *Anal. Bioanal. Chem.* 391, 2265-2276.
- Hernández, F., M. José, O. J. Pozo, J. V. Sancho, F. J. López, and I. Morell (2008). Pesticide residues and transformation products in groundwater from a Spanish agricultural region on the Mediterranean Coast. *Inter. J. Environ. Anal. Chem.* 88, 409-424.
- Ibáñez, M., O. J. Pozo, J. V. Sancho, J.V., F.J. López, and F. Hernández (2005). Residue determination of glyphosate, glufosinate and aminomethyl phosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry. *J. Chromatogr. A.* 1081, 145-155.
- Jacobsen, C.S., P. van der Keur, B.V. Iversen, P. Rosenberg, H. C. Barlebo, S. Torp, H. Vosgerau, R.K. Juhler, V. Ernstsén, J. Rasmussen, U. C. Brinch, and O. H. Jacobsen(2008). Variation of mcpa, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons. *Environ. Pollut.* 156, 794-802.
- Jiang, B., C. Yao, H. Wang, K. Gao, B. Lv, L. Jiang, R. He, and W. Pan(2011). Study on Relationship between Microbial Diversity and Organophosphate Pesticide Residues in Planting Base Soils of Shenyang. *Proc. ICICTA.* proceeding pp. 878-881.
- Juhler, R., S. Sørensen, and L. Larsen(2001). Analysing transformation products of herbicide residues in environmental samples. *Water Research* 35, 1371 – 1378.
- Katagi, T. (2013). Soil column leaching of pesticides. *Rev. Environ. Contam. Toxicol.* 221, 1-105.
- Kern, S., H. Singer, J. Hollender, R. P. Schwarzenbach, and K. Fenner(2011). Assessing Exposure to Transformation Products of Soil-Applied Organic Contaminants in Surface Water: Comparison of Model Predictions and Field Data. *Environ. Sci. Technol.* 45, 2833-2841.
- Kenneth, R. D. (1990). Pesticides in the Soil Microbial Ecosystem. *Enhanced Biodegradation of Pesticides in the Environment.* Am. Chem. Soci., pp. 1-12.
- Kolpin, D. W., E. M. Thurman, and A. D. Goolsby (1996). Occurrence of Selected Pesticides and Their Metabolites in Near-Surface Aquifers of the Midwestern United States. *Environ. Sci. Technol.* 30, 335-340.
- Lagana, A., G. Fago, A. Marino, and V. M. Penazzi (2000). Liquid chromatography mass spectrometry tandem for multiresidue

- determination of selected post-emergence herbicides after soil column extraction. *Analytica Chimica Acta* 415, 41-56.
- Landry, D., D. Sylvie, J.C. Fournier, and F. Andreux (2005). Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards (Vosne-Romanee, 21-France). *Environ. Pollut.* 138, 191-200.
- Mamy, L., E. Barriuso, and G. Benoit (2005). Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops. *Pest Manag. Sci.* 61, 905-916.
- McLaughlin, R. A., J. B. Weber and R. L. Warren (1994). Protecting Groundwater in North Carolina, a pesticide and soil ranking system. 439-31.
- Mosleh, Y.Y., S. P. Palacios, M. Couderchet and G. Vernet (2003). Effects of the herbicide isoproturon on survival, growth rate, and protein content of mature earthworms (*Lumbricus terrestris* L.) and its fate in the soil. *Applied Soil Ecology* 23, 69-77.
- Mudd, P., R. Hance, and S. J. L. Wright (1983). The persistence and metabolism of isoproturon in soil. *Weed Research* 23, 239-246.
- Nawab, A., A. Aleem, and A. Malik (2003). Determination of organochlorine pesticides in agricultural soil with special reference to γ -HCH degradation by pseudomonas strains. *Bioresource Technol.* 88, 41-46.
- OECD. (1993). OECD guidelines for the testing chemicals: leaching in soil columns. Paris.
- Osano, O., W. Admiraal, and D. Otieno (2002). Developmental disorders in embryos of the frog *Xenopus laevis* induced by chloroacetanilide herbicides and their degradation products. *Environ. Toxicol. Chem.* 21, 375-379.
- Polati, S., M. Bottaro, P. Frascarolo, F. Gosetti, V. Gianotti, and M. C. Gennaro (2006). HPLC-UV and HPLC-MSn multiresidue determination of amidosulfuron, azimsulfuron, nicosulfuron, rimsulfuron, thifensulfuron methyl, tribenuron methyl and azoxystrobin in surface waters. *Anal. Chimica Acta* 579, 146-151.
- Richardson, S.D. (2004). Environmental mass spectrometry: Emerging contaminants and current issues. *Anal. Chem.* 76, 3337-3364.
- Sinclair, C.J., and A. B. A. Boxall (2003). Assessing the ecotoxicity of pesticide transformation products. *Environ. Sci. Technol.* 37, 4617-4625.
- Sinclair, C.J., Boxall, A.B.A., Parsons, S.A., M. R. Thomas (2006). Prioritization of Pesticide Environmental Transformation Products in Drinking Water Supplies†. *Environmental Science & Technology* 40, 7283-7289.
- Sørensen, S.R. and J. Aamand (2001). Biodegradation of the Phenylurea Herbicide Isoproturon and its Metabolites in Agricultural Soils. *Biodegradation* 12, 69-77.
- Spliid, N.H. (2002). Leaching of pesticides from fields with recorded pesticide applications. *Intern. J. Environ. Anal. Chem.* 82, 571-582.
- Sprankle, P., W.F. Meggitt, and D. Penner (1975). Adsorption, mobility, and microbial degradation of glyphosate in the soil. *Weed Science* 23, 229-234.
- Stevens, M. and T. Duxbury (1992). *Aspergillus niger* and a *Penicillium* sp. are not directly involved in the degradation of chlorsulfuron. *Pesticide Sci.* 36, 287-291.
- Walker, A., and S. J. Welch (1992). Further studies of the enhanced biodegradation of some soil-applied herbicides. *Weed Research* 32, 19-27.