POSTER SESSION 7C

HERBICIDES: THEIR FATE, PERSISTENCE, DISTRIBUTION AND ECOTOXICOLOGICAL IMPACT

Session Organiser	Professor J C Hall *University of Guelph, Canada*
Poster Papers	7C-1 to 7C-12

Herbicide surface runoff and leaching from a cotton-rye cropping system under contrasting tillage and nutrient management levels

W K Vencill, D E Radcliffe, M L Cabrera
Crop and Soil Sciences, University of Georgia, Athens, GA 30602 USA

D Endale, J L Steiner, H H Schomberg
USDA-ARS, Watkinsville, GA 30677 USA

L L Lohr
Agricultural Economics Department, University of Georgia, Athens, GA 30602 USA

ABSTRACT

Field studies were conducted in 1997 and 1998 to examine fluometuron and pendimethalin surface runoff and leaching after application to cotton under two tillage (conventional- and no-tillage) and fertility regimes (synthetic fertilizer and broiler litter). Fluometuron was detected in surface runoff and leachate for approximately 40 days after application. Fluometuron was detected at higher levels in no-tillage than conventional-tillage cotton and cotton treated with broiler litter. Differences in fluometuron concentration in leachate amongst tillage and fertility treatments were not observed. The desmethyl metabolite was identified in all water samples containing fluometuron. Pendimethalin was not detected in surface runoff or leachate. Due to extended dry conditions, surface runoff and leachate samples were not collected in 1998.

INTRODUCTION

Concomitant with increased cotton acreage has been an increase in the use of cotton pesticides. Fluometuron is a substituted-urea, broadleaf herbicide applied to soil and/or foliage on about 70% of all cotton planted in the U.S. (Gianessi, 1991). Because of their moderate solubility in water, substituted-urea herbicides like fluometuron may leach readily through the root zone, especially in regions, like the Southeast, which are dominated by well-drained, low organic matter soils. Pendimethalin is a dinitroaniline herbicide that is soil-applied for control of annual grass and broadleaf weeds on about 30% of all cotton planted in the U.S. (Gianessi, 1991). Because of their low water solubility, dinitroanilines such as pendimethalin are resistant to leaching. However, they may be transported with eroded soil to surface streams.

Because it is rich in plant nutrients, broiler litter is commonly utilized/disposed of by spreading it on agricultural land (Simpson, 1991), usually pastures. Expansion of cotton acreage in the Southeast provides an additional outlet for broiler litter utilization, and its potential for use in cotton production has generated considerable interest among farmers in the region. Since it is a complex organic material, broiler litter may affect greatly the persistence and mobility of pesticides. However, direct and conclusive evidence is lacking. Broiler litter may accelerate degradation of pesticides by enhancing biological activity (Bollag & Liu, 1990), reducing the quantity of pesticide present for transport. Insoluble and particulate fractions of broiler litter may adsorb pesticides (Koskinen & Harper, 1990), tying pesticide fates to their own (at least for a while). Soluble organic fractions in broiler litter may complex with pesticides, enhancing pesticide solubility and mobility in leachate and runoff (Senesi, 1993). Because tillage affects the distribution and disposition of broiler litter in the field, tillage is expected to affect the magnitude of these processes, and their summed effect on pesticide loss.

With recent, rapid growth in cotton acreage, continuing expansion and concentration of a large poultry industry as well as increasing adoption of no-tillage practices, there is the potential for serious water quality degradation in the Southeast. Addressing these factors simultaneously in fully integrated farming systems offers the potential to create new strategies for disposing of poultry litter, controlling nutrient, pesticide runoff and leaching in a manner that is cost-effective.

MATERIALS AND METHODS

The research was conducted at the Water Quality Research Site (WQRS) at the USDA-ARS Southern Piedmont Conservation Research Center in Watkinsville, GA. Soil at the site is Cecil sandy loam (clayey, kaolinitic, thermic Typic Kanhapludults). The experimental design was a completely randomized block with a factorial combination of tillage (conventional- vs. no-tillage) and primary fertility source (broiler litter vs. chemical fertilizer). Each treatment combination was replicated three times. The experimental site consisted of 12 plots with the dimensions of 10 m by 30 m. Each of the twelve plots contained five, 30-m length drain lines spaced 2.5 m apart. The drain lines were flexible, slotted 10-cm diameter PVC tiles, installed on a 1 % grade. The depth of the tile at the end of the drain lines were approximately 1 m. The bottom of each trench in which tile-drains were installed was lined with 4-mil polyethylene plastic. The collection barrels were 1.5-m diameter steel culverts cut to a length of 2 m and set in the ground vertically. Each barrel accepts drainage from two plots, but the drainage from each plot is collected, stored, and analyzed separately. Drain lines enter the collection barrel at 1.2 m below the soil surface. Tipping buckets, fabricated of stainless steel, were used to measure the volume of water draining from the plots. Two buckets measuring 30.5-cm wide by 35.6-cm long were separated by a 0.65-cm sampling slot. The effluent captured as the sampling slot crosses the drain-line stream is directed into a 750-ml reservoir. The number of "tips" were recorded via an encapsulated reed switch. Sequential sampling of the effluent throughout a drainage event is achieved by interfacing the encapsulated reed switches to an ISCO model 3700 FR sequential waste-water sampler and a Campbell Scientific CR7 data logger. Each tip is directly proportional to a known volume, and the sampler is calibrated to subsample at pre-determined volume intervals. When the sampler is activated, a peristaltic pump empties the 750-ml reservoir in the collection barrel into a 1000-ml container in the ISCO sampler. Sample temperature was maintained at 2^0C by the ISCO unit to prevent its degradation. The drainage volume was calculated hourly from the number of tips recorded by the data logger. A runoff collector (14 gauge galvanized steel) spans the plot width (10 m) at the base of each plot. Each collector concentrates the surface runoff from a single plot, and channels it to a recording HS flume, where water height was measured and recorded continuously during an event to provide runoff volume. Each HS flume discharges onto a Coshocton wheel, where the runoff was subsampled for analysis of sediment load and solute concentration. As is the case of drainage samples, runoff samples were collected sequentially, and stored under refrigeration (2^0C) in the field by ISCO Model 3700 FR wastewater samplers, while they awaited removal for analysis.

The conventional tillage treatment consisted of chisel plowing and disking for incorporation of herbicides, broiler litter and fertilizer, and for seedbed preparation. The no-tillage treatment consisted of coulter planter use only.

The broiler litter treatment contained 4.5-Mg broiler litter ha^{-1}. The chemical fertilizer treatment was according to University of Georgia recommendations, which consist of preplant application of 22.5 kg N ha^{-1} (as NH_4NO_3,) and P and K fertilizer (as indicated by soil testing), and an application of an additional 45 kg N ha^{-1} (as NH_4NO_3) when cotton was at the first square stage of development. Previous research has indicated the desirability of using non-leguminous winter cover crops as a management practice that reduces erosion and associated nutrient transport (Sharpley & Smith, 1991) and limits nitrate leaching (McCracken *et al.*, 1993). Wheat was sown on all plots each fall.

Fluometuron and pendimethalin were uniformly surface-applied at the rate of 1.1 kg a.i. ha^{-1} on 10 May 1997 and 11 May 1998. Broiler litter (4.5 Mg ha^{-1}) and N-P-K fertilizer (22.5 kg N ha^{-1} as NH_4NO_3, and soil test recommendations for P and K) treatments were broadcast, along with supplemental nutrients as required. Herbicides and nutrient sources were soil-incorporated with conventional tillage, or left on the surface with no-tillage.

Throughout the year, leachate and runoff samples were collected continuously as drainage and surface flow occurs. Samples were collected and stored under refrigeration (2^0C) in the field by ISCO model 3700 FR sequential wastewater samplers, until removed for analysis. Micromembrane filtration was used to separate sediment from runoff for analysis. Methanol was be used to extract pesticides from soil.

Herbicides were extracted from water samples using C_{18} solid phase extraction disks. The volume of water (300 ml) was processed through a pre-treated disk under vacuum. The sample was removed from the extraction disk with hexane. Sample volume was concentrated to 1 ml in hexane. Sample recovery was greater than 90%.

Fluometuron, and pendimethalin in soil, leachate and runoff were quantified by gas chromatography/ mass spectrometry (HP 6890GC/5973MSD). A 5 ul aliquot of sample was automatically injected on a split-splitless system for analysis by GC/MS on a 20 m x 0.25 μM HP-5 MS phenyl methyl siloxane capillary column. Operating temperatures were a program ramp of 80^0C/1 min; 178^0C/4min; 205^0C/15 min; and 310^0C/4 min, inlet temperature of 250^0C, and mass spectrometer was set at a source temperature of 210^0C and quadrupole temperature of 140^0C. Carrier gas was helium at 50 mL min^{-1}.

RESULTS AND DISCUSSION

Figure 1 shows the rainfall patterns for the 1997 and times of detectable surface runoff of precipitation.

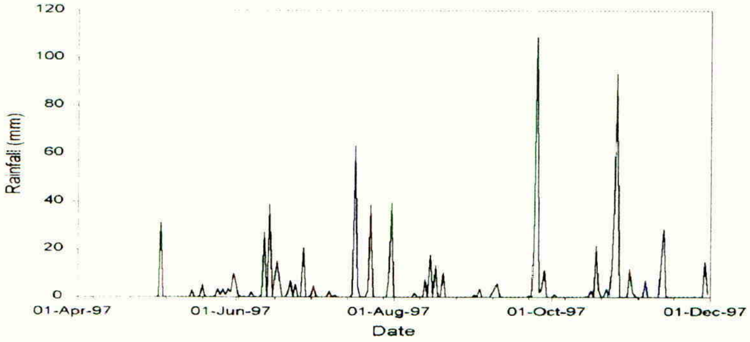


Figure 1. Precipitation in 1997.

In 1997, the first rainfall was 12 days after herbicide application. After that precipitation event, enough precipitation was received to collect runoff on 12 June, 17 June, 17 July, 31 July, and 26 September. The time lag between herbicide application and first rainfall may account for low levels of fluometuron and pendimethalin in leachate and surface runoff.

Pendimethalin was detected in one plot for the 1997-growing season. The low water solubility (0.275 mg L^{-1}) high K_{ow} probably accounts for the lack of pendimethalin movement in surface runoff or leachate water. Fluometuron concentrations in surface runoff were greater than those detected in leachate (Figure 2).

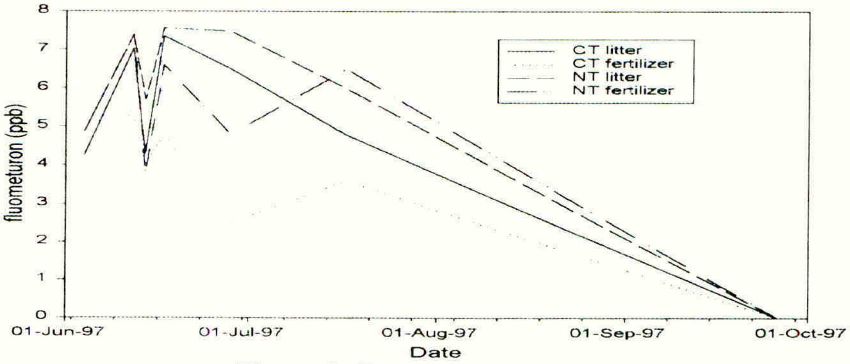


Figure 2. Runoff of fluometuron.

Fluometuron was not detected in surface runoff from any plot 40 days after application. Fluometuron concentrations were greater in no-tillage cotton than in conventional tillage cotton. Fluometuron concentrations were also greater in plots treated with broiler litter than those treated with synthetic fertilizer. A possible explanation for these differences could be attributed to greater levels of organic matter moved off site with surface runoff in the both no-tillage cotton and cotton treated with broiler litter. Willian *et al.* (1997) reported that fluometuron half-life in the 0- to 8-cm zone of the soil was 9 to 28 days and fluometuron half-life was inversely correlated with organic matter. Thus, fluometuron would be expected to reamain longer in the treatments with greater organic matter such as no-tillage plots and those treated with broiler litter. The desmethyl metabolite of fluometuron was detected in all water samples containing fluometuron. Fluometuron concentrations were lowest in surface runoff water in conventional cotton treated with synthetic fertilizer.

Fluometuron was not detectable in surface runoff or leachate 40 days after application (Figure 3).

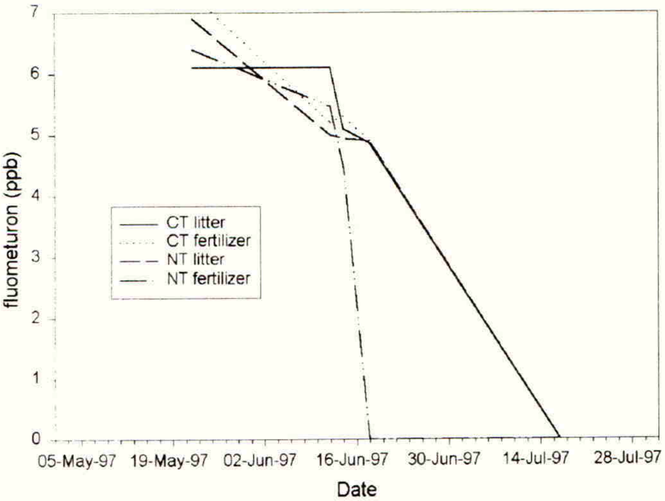


Figure 3. Detectable fluometuron in leachate.

Fluometuron concentrations were greater in leachate from conventional-tillage cotton than no-tillage cotton. The desmethyl metabolite was detected in all water samples containing fluometuron. Differences were not observed between cotton treated with broiler litter and synthetic fertilizer.

In 1998, precipitation was not great enough to collect water samples from surface runoff or leachate during the growing season due to an extended drought.

ACKNOWLEDGEMENTS

This work was supported by NRI Water Quality Program. The authors thank Maria Monteros for technical assistance.

REFERENCES

Bollag J M; Liu S Y (1990). Biological transformation processes of pesticides. In: *Pesticides in the Soil Environment: Processes, Impacts, and Modeling*, ed H H Cheng, pp. 169-211. Soil Science Society of America, Madison, WI.

Gianessi L; Puffer C A (1991). *The Use Of Herbicides In U.S. Crop Production: Use Coefficients Listed By Active Ingredient.* Resources for the Future, Washington, D.C.

Koskinen W C; Harper S S (1990). The retention process: Mechanisms. In: *Pesticides In The Soil Environment: Proceses, Impacts, And Modeling, ed* H H Cheng, pp. 51-77. Soil Science Society of America, Madison, WI.

McCracken D V; Hargrove W L; Box J E; Cabrera M L; Johnson J W; Raymer P L; Harbers G W; Johnson A D (1993). Influence of tillage and cover cropping on nitrate leaching. In: *1993 Southern Conservation Tillage Conference For Sustainable Agriculture.* ed P K Bollich, pp. 11-15. Louisiana State University Agricultural Center, Baton Rouge, LA.

Willian W T; Mueller TC; Hayes R M; Snipes C E; Bridges D C (1997). Adsorption, dissipation, and movement of fluometuron in three southeastern United States soil. *Weed Science* **45**, 183-189.

Senesi N (1993). Organic Pollutant Migration In Soils As Affected By Soil Organic Matter: Molecular And Mechanistic Aspects. In: *Migration And Fate Of Pollutants In Soils And Subsoils,* ed D Petruzzelli & F G Helfferich. pp. 47-74. Springer-Verlag, New York.

Sharpley A N; Smith S J (1991). Effects of cover crops on surface water quality. In: *Proceedings International Conference Cover Crops For Clean Water,* ed W L Hargrove , pp. 41-49. Soil and Water Conservation Society, Ankeny, IA.

Simpson T W (1991). Agronomic use of poultry industry waste. *Poultry Science* **70**, 1126-1131.

Factors affecting the loss of six herbicides from hard surfaces

A J Shepherd, A I J Heather
Soil Survey and Land Research Centre, Shardlow Hall, Shardlow, Derby DE72 2GN, UK

ABSTRACT

Studies of the environmental fate of herbicides have focused almost exclusively on the agricultural environment. Here we report initial results from a series of studies investigating the fate of six herbicides (glyphosate, isoxaben, oryzalin, oxadiazon, diuron and atrazine) with varying physico-chemical properties, applied to different types of man-made surfaces, asphalt, concrete and ballast. Losses of each herbicide were studied under replicated, semi-controlled environmental conditions. Following herbicide application, simulated rainfall was applied at varying rates and different time-lag intervals. All surfaces were new and free from weed infestations. Results indicated that, for all but one compound, accumulated wash-off (% AS) was significantly different for all three surfaces, concrete being the worst. In general, a 5-mm rainfall event produced significantly less accumulated losses than events of 10- and 15-mm. With the exception of one compound (glyphosate), time-lag events had no significant effect on losses from asphalt. In contrast, significant differences were recorded between time-lag events for five of the compounds, on ballast. On concrete, three of the six compounds showed significantly smaller accumulated losses after a 6-h time-lag compared to those of 12-, 24- and 168-h, and two showed significantly greater losses after the168 hour interval.

INTRODUCTION

Control of weeds on non-agricultural surfaces such as pavements, roads edges and railways is common practice. Weeds deform surface structures and compromise both safety and aesthetics. At present, herbicides are the only feasible method of control, but their use has led to concerns over the extent to which such compounds are detected in surface and ground water in and around urban areas. To provide preliminary data on herbicide removal, a pilot study on a road edge was undertaken (Heather *et al.*, 1998). The study identified a number of factors that may be important in determining the loss of herbicides from a specific type of man-made surfaces. However, the information and data generated was limited. This study, involving controlled wash-off experiments, was therefore performed to confirm that the identified factors did indeed have an influence on herbicide loss. The main objectives of the study were to determine the:

1. relationship between surface type, physico-chemical properties of a range of herbicides and surface wash-off characteristics;
2. effects of rainfall rate on the nature of herbicide wash-off from hard surfaces;
3. effects of rainfall duration on the nature of herbicide wash-off from hard surfaces; and
4. effects of time-lag between herbicide application and rainfall on the nature of herbicide wash-off from hard surfaces

MATERIALS AND METHODS

Six active substances were selected that had a range of physico-chemical properties (Table 1). Compound choice was also influenced by previous studies. Products containing the actives substances were obtained directly from the agrochemical companies. Application rates and water volumes for the test compounds were based on specific label information for amenity areas, where possible this represented typical use.

Table 1. Product information and physico-chemical properties for the study compounds.

Compound	Glyphosate	Isoxaben	Oryzalin	Oxadiazon	Diuron	Atrazine
Formulation	SL	SC	SC	WP	SC	SC
A.S. g L^{-1}	360	125	480	300*	500	500
K_{OC}	10000	1400	700-1100	3200	480	100
Soil DT_{50} (21°C) days	47	90-120	63	60	90-180	35-50
Solubility (water) mg L^{-1}	11600	1.42	2.6	10	36.4	33
Product application rate (L ha^{-1})	5	0.6	3.6	15**	5.4	6
Water volume (L ha^{-1})	400	400	400	450	450	450

*	g kg^{-1}	SL: Soluble Concentrate
**	kg ha^{-1}	SC: Suspension Concentrate
A.S.	active substance	WP: Wettable Powder

New surface material was used in order to remove interfering factors such as surface weathering and to allow comparison of results. The asphalt sample surfaces were constructed, using moulds, from a wearing course grade of asphalt. Pre-pressed paving slabs formed the concrete samples. The ballast sample surfaces were constructed from 50-mm granite contained in a 600 x 900 x 400 mm mild steel bin with provision for drainage. All test surfaces had a surface area of 0.54 m^2.

The herbicides were applied individually using a modified hand spray gun. Two treatment groups were used. Thus, each surface received an application of three individual compounds. The treatment groups were (1) glyphosate, isoxaben and oryzalin and (2) oxadiazon, diuron and atrazine. All surfaces and compound applications were replicated three times. After application, all surfaces were exposed randomly (within groups) to a natural climate (mean temperature: 8.5 °C), for either 6-, 12-, 24- or 168-h. During the exposure period, no natural rainfall was allowed to make contact with the surface.

Following the designated exposure period, each surface was transported indoors and placed on a surface stand, under a rainfall boom. Surface stands were built with a gradient of 10% to allow surface water flow. Rainfall events of 5-, 10- or 15-mm were then simulated. The varying volume of rainfall applied was achieved by a pulsing on/off cycle. Water used for rainfall simulation was from the tap and had a pH of 6.9 – 7.9.

Under irrigation, wash-off samples were collected using a gutter funnel arrangement. Three samples from the beginning, middle and end of the rainfall event were taken from each surface. For one event, a sequential sampling sequence was adopted. All samples were stored at $4^{O}C$ in a refrigerator. On completion of the study, the glyphosate samples were sub-sampled and the study samples placed in plastic bottles and frozen. Samples were sent to the analytical laboratories in freezer boxes to minimise degradation of any active substances present during transport.

RESULTS

In general, concentrations of herbicides removed were greater from concrete than from asphalt or ballast. The exception to this was glyphosate, where concentrations were greater in run-off from asphalt. For all compounds on asphalt and concrete highest concentrations were observed in samples collected after < 1-mm of rainfall. On concrete, the peak concentration was significantly higher than from asphalt. Run-off from ballast was significantly slower than from asphalt or concrete and consequently peak herbicide concentrations were not detected in the first 1mm of rainfall. The maximum detected concentration for concrete surfaces was 119.68 mg L^{-1} for atrazine. The maximum concentration from asphalt surfaces was 137.0 mg L^{-1} for glyphosate. On ballast, the maximum concentration observed was 16.73 mg L^{-1} for atrazine.

On asphalt, in all cases, the 5-mm rainfall event produced significantly lower total loss, than events of 10- and 15-mm ($P < 0.05$). Highest total losses were recorded for glyphosate of 54.9 % following the 15-mm event. Relative total loss for all other compounds on asphalt in relation to glyphosate for the 15-mm event were: atrazine > isoxaben > diuron > oryzalin > oxadiazon

On concrete, losses of all six of the active substances were significantly lower following the 5-mm rainfall event than those generated by the 10- and 15-mm events ($P < 0.05$). The maximum-recorded total loss on concrete was observed for isoxaben following the 15-mm rainfall event. Relative losses for all other compounds on concrete in relation to isoxaben for the 15-mm event were: atrazine > glyphosate > diuron > oryzalin > oxadiazon

For four of the six compounds on ballast, the rainfall event of 5-mm produced significantly lower total loss than events of 10- and 15-mm for four of the six test compounds. For isoxaben and oxadiazon, different rainfall events had no effect on total loss. Rank order of loss from ballast was: atrazine > diuron > isoxaben > glyphosate > oryzalin > oxadiazon

Total losses for all compounds and surface types following time-lag events of 6-, 12-, 24- and 168 h are shown in Figure 1. On asphalt, time-lag had no effect on wash-off for five of the test compounds. Glyphosate was the exception, with the 6-h time-lag producing significantly higher percentage loss than events of 12-, 24-, and 168-h ($P < 0.001$). The highest total loss of 49% from 6-h time-lag event was recorded for glyphosate. Relative losses for all other detected compounds in relation to glyphosate were: atrazine > isoxaben > diuron > oryzalin > oxadiazon

On concrete surfaces, for three of the compounds, isoxaben, oryzalin and atrazine, the 6-h time-lag resulted in significantly lower losses than any other time-lag event ($P < 0.001$). For oxadiazon and diuron, an interval of 168-h resulted in a significantly higher loss than other time-lag events. For the 168-h event, isoxaben showed the highest total loss. Relative losses for all other compounds in relation to isoxaben for the 168-h event were: atrazine > oryzalin > diuron > oxadiazon > glyphosate

Total losses on ballast, for four of the compounds increased with increasing time-lag intervals. For glyphosate and oxadiazon, the factor of time-lag made little difference to the total loss.

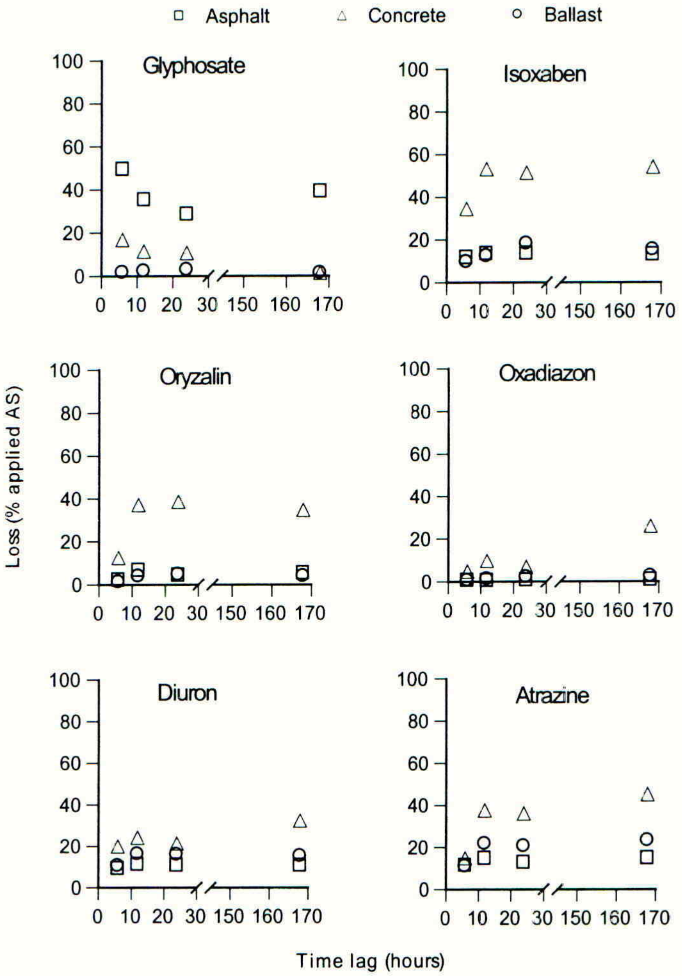


Figure 1. Total loss from asphalt, concrete and ballast following 6-, 12-, 24- and 168-h time-lag between herbicide application and 5-mm rainfall event.

DISCUSSION

There was a marked difference between surface type and rate of herbicide removal (Figure 2). In general, losses of herbicide from concrete were greatest; the exception to this was glyphosate where the highest loss was observed for asphalt. Concrete is less pitted and less permeable than asphalt or ballast, and probably has a much lower surface area available for sorption than the other surfaces. The observations for glyphosate are possibly due to its dissociation potential, the negatively charged species binding to the cationic calcium on concrete.

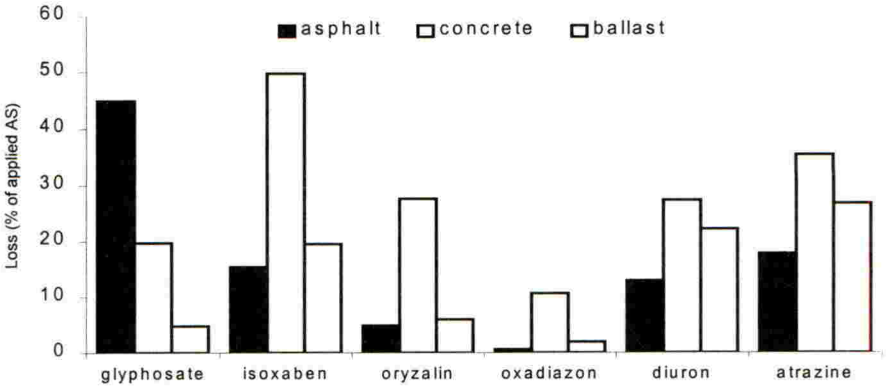


Figure 2. Mean total loss of each herbicide from asphalt, concrete and ballast.

The studies were performed using new surfaces. Due to minimal weed infestation, new surfaces are unlikely to receive applications of herbicide. Furthermore, organic material will normally accumulate on hard surfaces over time. As several of the herbicides are likely to partition to organic matter, this could result in increased adsorption. Studies with new surfaces should however provide a worst case indication of environmental exposure.

The time for runoff to be initiated varied between the different surface types. On concrete, runoff occurred 3 minutes after a 5-mm rainfall event whilst for asphalt, runoff occurred 7 minutes after the 5-mm event. The time for runoff from ballast to be initiated was much slower, and may be explained by the depth of ballast used to construct the surfaces. Spot samples indicated that for both concrete and asphalt the majority of herbicide loss occurred after < 0.66 mm of accumulated rainfall. Continuous monitoring supported these observations and demonstrated that herbicide loss reaches a steady state after 2-mm rainfall.

Rainfall duration influenced herbicide loss; a longer rainfall event resulting in higher loss. Consequently, although most losses occurred during a 1- to 2-mm rainfall event, accumulated losses from longer events would be higher. The slower run-off rate for ballast meant that it was difficult to interpret these results. However, as with asphalt and concrete surfaces it is likely that the length of rainfall duration will effect loss from ballast.

Rainfall rate did effect the level of herbicide wash-off from hard surfaces; 5-mm of rainfall resulting in less herbicide loss than the 10 and 15-mm rainfall events for all surface types. For asphalt, comparison of the 5-mm end sample and 10-mm middle sample indicated that the results could be explained purely on the basis of volume of water applied. For concrete, whilst loss was also effected by rainfall rate, for four of the six compounds the effects could not be explained by difference in volume of water applied. Consequently, 5-mm of rainfall falling in 30 minutes may result in greater losses than 5-mm falling in 70 minutes. On ballast, for four of the six test compounds, loss increased with increasing rainfall. The effects of time and water volume could not be investigated, so the results could only be expressed as a total loss figure.

Time-lag events of 6-, 12-, 24- and 168-h did have an effect on loss, however the effect was not the same for all compounds. On concrete, only glyphosate behaved as expected, with loss decreasing with increasing time-lag. Conversely, the losses of oxadiazon and diuron were highest following a time-lag interval of 168-h. For isoxaben, oryzalin and atrazine a time-lag interval of 12-, 24- or 168-h resulted in higher losses than a time-lag of 6-h. Therefore, for five of the test compounds, losses will increase with increasing time-lag interval between compound application and rainfall. Generally, losses from ballast after 12-, 24- and 168-h time-lag were greater than losses after a 6-h time-lag. The exception to this was glyphosate where time-lag had no significant effect on herbicide concentrations.

The relationships between physico-chemical properties (K_{OC}, DT_{50} and solubility) and wash-off were investigated using a correlation matrix. For soil K_{OC}, no strong trends were observed, with both positive and negative relationships seen for each surface type. Likewise, the relationship between water solubility and loss was also unclear. On asphalt, there was a strong positive relationship between loss and solubility, thus highly soluble compounds will have higher loss. However, this trend was not present for concrete or ballast, unless glyphosate was removed from the matrix. Glyphosate has a water solubility value of 11600 mg L^{-1}, all other compounds tested in this study have values < 37 mg L^{-1}. With glyphosate excluded, the significance of the positive relationship was only evident for ballast. No clear significant relationship existed between DT_{50} and wash-off, regardless of influencing factors or surface types. This lack of correlation between total losses and soil DT_{50}, for the compounds suggest that microbial degradation is not a significant factor.

CONCLUSION

Regardless of influencing factors, herbicides do seem to be readily removed from hard surfaces. Both surface and herbicide type determine which factors effect losses, given that losses remain relatively similar under varying rainfall and time-lag events. In all cases, water volume plays a significant part in herbicide removal, with relatively small volumes of water removing a large proportion of applied herbicide, especially in the case of asphalt and concrete. A combination of influencing factors were involved in the movement of herbicide from hard surfaces. The effect of these factors will be determined by the herbicide and the hard surface type.

ACKNOWLEDGEMENTS

The authors acknowledge the support of Department of the Environment Transport and the Regions, Dow Agrosciences, Monsanto Agricultural Company for funding this research.

REFERENCES

Heather A I J; Shepherd A J; Hollis J M (1998). Losses of six herbicides from a kerb and gully pot road drain. Silsoe: Cranfield University, Soil survey and Land Research Centre (unpublished report).

Fate and behaviour of pesticides in farm ditches

R J Williams, C White, S Dreymann
Institute of Hydrology, Wallingford, Oxfordshire, UK

V Gouy, C Garon-Boucher, C Souiller
Cemagref, 3 bis Quai Chauveau, 69336, Lyon, France

ABSTRACT

Cemagref in France and the Institute of Hydrology in the UK have recently established experiments to determine the role played by ditches in altering pesticide concentrations leaving fields and arriving at permanent water courses. In France, isoproturon and diflufenican were introduced into an experimental ditch at concentrations similar to those observed in runoff events. Both pesticides concentrations were reduced compared to a chloride tracer, 54% and 27% after 50 m for isoproturon and diflufenican, respectively. In the UK, natural ditches have been monitored for pesticides applied to the catchment draining to two ditches and concentrations of the herbicide propyzamide monitored down the ditch. Laboratory experiments suggested that, after 55 days, there was no degradation potential for isoproturon in the ditch water, but that sorption to bed sediments was a likely mechanism for loss of the pesticides.

INTRODUCTION

Extensive research has been carried out on pesticide losses from plots and fields (e.g. Burgoa & Wauchope, 1995). The processes that lead to pesticide runoff are reasonably well understood although they have proved too complex to model reliably at present. There have been some studies of small catchments (e.g. Williams *et al.*, 1995) and one large catchment study in the UK (Clark *et al.*, 1991). However, the relationship between these measured loads and those generated at the edge of fields has not been established. A pesticide leaving a field in runoff may pass through one or more environments before it reaches a major river including; another field, a ditch, a pond and various order streams. The amount of pesticide passing through these environments may be modified by a number of physical, biological and chemical processes: sorption (to sediments or plants), volatilization, uptake (by plants and animals), degradation (biotic and abiotic), release from sediments. The contribution made by each of these processes to the reduction (or increase) in loads of pesticide transported is not well known for any of the environments listed above (except for perhaps ponds). This paper describes the approach and preliminary results from two, parallel projects, studying 4 herbicides (Table 1). The experiments were designed to improve our understanding of the role of ditches in the transport of pesticides to the main river network

METHODS

Experiments are being conducted at two sites, the Oxford University Farm at Wytham, Oxfordshire and at Le Cétrais near Nantes in the West of France by Cemagref in collaboration with the Chambre d'Agriculture de Loire Atlantique.

Table 1. Properties of the herbicides studied in the field and laboratory experiments.

Characteristics	Isoproturon	Diflufenican	Propyzamide	Trifluralin
Vapour pressure (mPa)	0.0033 (20°C)	0.07 (30°C)	0.058 (25°C)	9.5 (25°C)
Koc (l/kg)	120	1990	800	8000
DT50 (soil, days)	12-32	175-294	21-90	60-132

The Le Cétrais site is located in a predominantly agricultural region dominated by cereal and maize production. The shallow loamy soils are tile drained and intersected by a dense network of ditches. The experiments were conducted on a single 50 m length of ditch which was initially dry and vegetated down a large part of its length with terrestrial species and an 8-cm layer of decomposing leaves. An artificial flow of 2.5 m/s of water was introduced into the ditch (0.25 m deep and 1 m wide at the surface) and maintained throughout the experiment. Once a steady flow had been obtained, an aqueous solution containing isoproturon, diflufenican and KCl was introduced over a period of 10 minutes at concentrations well below known toxicological thresholds. Sampling was undertaken 25 m and 50 m down from the injection point. Sampling, at 2 minute intervals, started when water conductivity increased (due to Cl^- arrival: 6 min at 25 m and 16 min at 50 m) and stopped when water conductivity returned to its initial value.

Samples were maintained at 7°C and analysed soon after sampling. A liquid-liquid extraction with dichloromethane was used for both pesticides. For diflufenican it was necessary to purify the extract on a florisil cartridge. Diflufenican was analysed by gas chromatography with an electron capture detector and isoproturon by liquid chromatography with an UV detector: Detection limits were 0.08 and 0.13 µg/l respectively (Patty & Guyot, 1995).

Wytham Farm is situated 10km west of Oxford, UK. The predominant soil type is heavy clay from the Denchworth series. The farm practices mixed agriculture based on sheep and cereal production. The cereals generally follow a 4-year rotation of 3 winter-sown cereals followed by winter sown oil seed rape. At Wytham, the aim is to study the behaviour of pesticides in ditches arising from runoff from fields receiving applications in the course of normal agricultural production. Two ephemeral ditches have been selected for study. Both ditches are vegetated down their entire length with terrestrial species (mostly grasses). The ditches are about 1-m wide and water flows at an average depth of ~0.25 m down a gradient of 2-3%. The ditches are fed by a combination of sub-surface drains, sub-surface lateral flow and some overland flow, although the latter is limited due to the presence of 3 m grassed buffer strips along the side of the ditches.

Initial sampling was carried out at the top, middle and bottom of both ditches at weekly intervals following pesticide application. For ditch B, propyzamide was applied to oil seed rape and trifluralin to winter wheat for ditch A. The sampling sites were chosen such that the application areas of these pesticides drained to the ditches above the topmost sampling sites.

At the time of writing this paper only the propyzamide data were available. Samples were stored at 4°C and extracted by C18 solid-phase cartridge within 48 hours of the sample being collected. The pesticides were eluted from the cartridges with acetonitrile and analysis was by GC-MS operating in single ion mode.

As discussed above the main factors likely to remove pesticides from solution in ditches are degradation and sorption. The likely significance of these processes as loss mechanisms in the ditches at Wytham was studied using the pesticide isoproturon.

For the sorption studies, duplicate bed sediment and ditch water samples were collected from upper (A1), middle (A2) and lower (A3) sampling sites in ditch A. Fresh sediments were used having been stored at 4°C for the few hours before the experiment was started. The bed sediments were placed into 45 ml PTFE centrifuge tubes and sufficient 0.45 μm filtered ditch water was added to give 15 ml liquid to 5 g (dry weight) solid in each tube. The sorption was measured at 3 concentrations of isoproturon: 25, 50 and 100 μg/l. These concentrations were achieved by mixing appropriate amounts of ^{14}C labelled isoproturon and cold isoproturon stock solutions and adding them directly to the PTFE tubes. Three replicates were made for each concentration and sediment sample. After mixing, the tubes were placed on a shaker at 200 rpm for 20 hours at room temperature (20°C). The tubes were centrifuged for 10 minutes at 6000 rpm and 2.5 ml of liquid was taken from each tube, passed through a 0.45 μm PTFE filter into a Sterilin tube. Subsequently, three, 1-ml sub-samples were placed into scintillation flasks and mixed with 5 ml scintillant (Ultima Gold). Each sample was counted for 5 minutes with a scintilation counter (Beckmann LS 6500).

For the water degradation experiment, samples were collected from the middle reaches of both ditches (sites A2 and B2) in 1 litre brown glass bottles. The ditch water was stored at 4°C for 1 day before the experiment was started. For each sample three 50-ml aliquots were placed in sterilised plastic flasks and 0.25 ml of 20 mg/l isoproturon stock solution was added to each flask to give a final concentration of 100 μg/l. This procedure was repeated with three sterile flasks and sterile ditch water (by autoclave for 30 minutes at 15 psi). All the samples were incubated at 10°C for 50 days. At weekly intervals, 0.15 ml was taken from each flask with a pipette and then passed through a 0.45 μm PTFE filter into an HPLC flask. Samples were injected into an HPLC equipped with UV detection set at 220 and 240 nm.

RESULTS

The concentrations of the two herbicides and chloride 50 m downstream of their injection point in the ditch experiment at Le Cétrais are shown in Figure 1. The mass of chloride recovered 50 m below the injection point was equal to that which had been added, implying there was no loss of water from the system (e.g. through seepage into the bed). The mass balance for the pesticides, however, showed a decrease in pesticide recovery with distance down stream. 25 m down from the injection point 37% of the diflufenican and 12% of the isoproturon had been lost. After 50 m the losses were 54% for diflufenican and 27% for isoproturon.

At Wytham, propyzamide was applied to an oil seed rape crop to fields above site B1 on 1 December 1998. The concentrations observed down the length of Ditch B at various times following the application are shown in Figure 2. There is clearly a reduction in concentration down the ditch in the water samples taken relatively soon after the application of the herbicide. However, after three months the concentrations are similar at all points and may in fact be higher at the bottom of the ditch than at the top. The laboratory experiments showed some variation in the sorption coefficients (K_d) for isoproturon with the bed-sediments in Ditch A. The pattern was for increasing sorption down the length of the ditch with K_ds of 2.0 and 2.5 l/kg being measured at site A1, a value of 3.2 l/kg at site A2 and values of 5.1 and 7.0 kg/l at site A3.

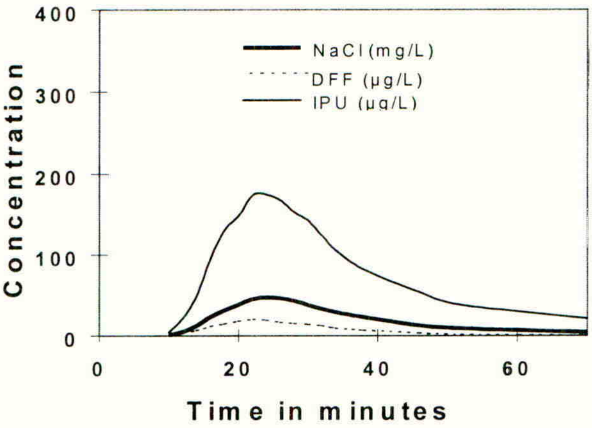


Figure 1. Pesticide and KCl concentrations in the ditch 50 m below the point of injection.

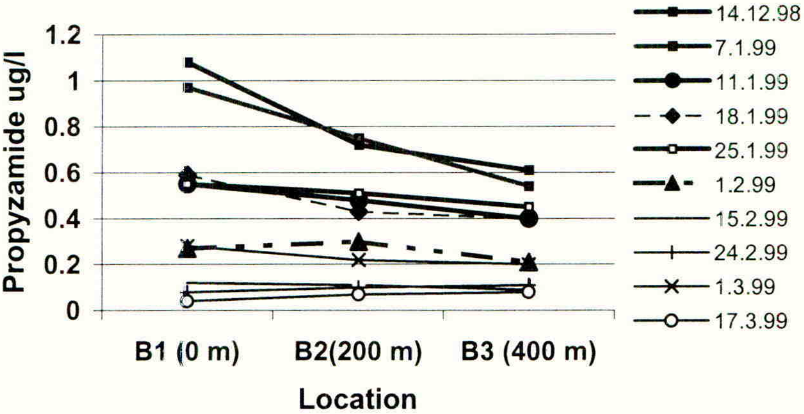


Figure 2. Concentrations of propyzamide at various times after application at three locations down Ditch B at Wytham, UK.

Isoproturon did not degrade in the ditch water during the period of the experiment (54 days). Figure 3 shows no changes in the concentrations of isoproturon in the incubated samples and no differences between the sterile and non-sterile flasks.

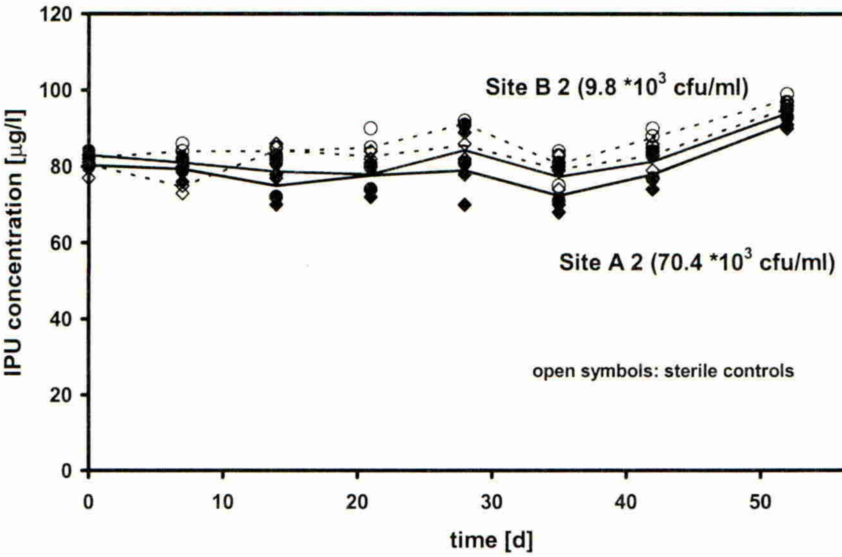


Figure 3. Concentration of isoproturon in water from Ditch A incubated at 10°C. The figures in brackets are the number of colony forming units counted at each site.

DISCUSSION

Evidence for reduction in pesticide concentrations down ditches is provided by the field data from both Wytham and Le Cétrais. At Wytham there is a 50% decrease in propyzamide down the ditch for samples taken shortly after its application. There was dilution of the pesticide by waters draining from untreated fields. At this stage in the study this dilution has not been measured so care must been taken in interpreting these data. At Le Cétrais under controlled conditions, the loss of diflufenican was noted to be greater than that for isoproturon. The half lives and K_{oc} values for these pesticides (Table 1) would suggest that diflufenican may be more recalcitrant and more sorptive than isoproturon. Since these properties should work in opposite directions in modifying pesticide concentrations, we may propose that sorption is the more important mechanism in pesticide attenuation in ditches during storm events. In any case, a residence time of less than 1 hour would not be sufficient for any degradation unless the reaction rate was very fast. If the laboratory incubation of isoproturon with Wytham ditch water is any indication of the likely rate of diflufenican degradation at Le Cétrais, then at best the expected rate would be very slow.

The Kd values measured for isoproturon and the bed-sediments in the upper reaches of Ditch A were very similar to those measured for adjacent fields (Johnson *et al*., 1996). This implies that the bed material is similar to the topsoil, at least in terms of the organic matter fraction. Moving down the stream the Kd values increase, which may indicate increasing organic fractions in the bed material originating from decaying leaf litter, etc. The affinity of isoproturon for bed-sediments confirms that sorption is likely to be an important process in ameliorating pesticide concentrations down ditches.

The data presented here are preliminary and further experiments at both sites are planned for the next 2 years. At Wytham, flow measurement and automatic sampling equipment have been placed on Ditches A and B and detailed mass balances of pesticide transport will be made. Measurements will also be made of pesticide concentrations in the bed-sediments. In the laboratory further studies of sorption and degradation will be made with pesticides in water, sediment and water/sediment systems. At Le Cétrais, more controlled experiments will be undertaken with other pesticide products at a range of flow rates and in consecutive events will be studied. The final objective of the project is to model of the fate and behaviour of pesticides in ditches in order to improve catchment scale estimates of pesticide runoff concentrations.

ACKNOWLEDGEMENTS

The work carried out by the Institute of Hydrology was part of the MAFF Pesticide Safety Directorate R&D programme (PL0518).

REFERENCES

Burgoa B; Wauchope R D (1995). Pesticides in runoff and surface waters. In: *Environmental behaviour of agrochemicals,* eds T R Roberts & P C Kearney pp 221-256, John Wiley and Sons: Chichester, UK.

Clark L; Gomme J; Hemmings S (1991). Study of Pesticide in Waters from a Chalk Catchment, Cambridgeshire. *Pesticide Science*, **32**, 15-33.

Johnson A C; Haria A H; Bhardwaj C L; Williams R J; Walker A (1996). Preferential pathways and their capacity to transport isoproturon in a structured clay soil. *Pesticide Scie*nce, **48**, 225-237.

Patty L; Guyot C (1995). Analytical methods for the determination of isoproturon and diflufenican residues in runoff and soil. Bulletin of Environmental Contamination and Toxicology. 55, 802-809.

Williams R J; Brooke D N; Matthiessen P; Turnbull A; Harrison R M (1996). Pesticide transport to surface waters within an agricultural catchment. *Journal of the Institution of Water and Environmental Management*, **9**, 72- 81.

Point-source pesticide contamination: quantification and practical solutions

S Higginbotham, R L Jones, E Gatzweiler
Rhône-Poulenc Agrochimie, Avenue René Cassin, Lyon, France

P J Mason
Coventry University, Priory Street, Coventry CV1 5FB, UK

ABSTRACT

The presence of pesticides in raw-water has been demonstrated through a number of monitoring programs. The routes by which pesticides enter water are known. However, to understand better the significance of these routes and to provide practical solutions, Rhône-Poulenc Agrochemie has conducted a number of catchment studies with isoproturon in Germany and the UK. These studies though demonstrating the generic mechanisms of surface run-off and macropore flow to tile drains, also show the importance of point sources in contributing to pesticides in surface water. In the UK, Rhône-Poulenc Agriculture is conducting a catchment study on the river Cherwell which illustrates the quantitative significance of the spray-yard activities of tank filling and cleaning. The study also demonstrates the significance of minute splashes of pesticides on levels of pesticides that appear in surface water.

This paper refers to these studies and reflects on the valuable contribution that the adoption of simple application practices will have in protecting raw-water. Many of the issues are generic in nature and the adoption of the practices could lead to a reduction in the amount of most pesticides found in surface water.

INTRODUCTION

The presence of pesticides in surface waters is well documented. Isoproturon (IPU) has been detected occasionally in groundwater and more frequently in surface water. Concentrations in untreated water rarely exceed aquatic toxicology standards (20 ppb) but have frequently exceeded the 0.1ppb drinking water standard. The mechanisms by which pesticides migrate to streams, rivers and reservoirs, whether by diffusion or from point sources is understood. However, to provide spray operators with well founded stewardship advice, it was necessary to quantify the relative importance of these mechanisms. Greater effort, in communicating to the spray operator, can focus on those practices that will be most effective at reducing pesticide levels in raw water.

Research to determine the timing and magnitude of isoproturon (IPU) losses from agricultural land to receiving water courses has focused, for the most part, on the post-spray period and attempted to assess the significance of diffuse surface and subsurface hydrological pathways. However, water quality monitoring data from 1995 provided by Thames Water and reviewed by the IPU Stewardship Group (U.K.), showed that IPU concentrations in the River Thames rose before the land drains began to flow. This rise, prior to a hydrological connection between treated agricultural soils and water courses, suggests that other agricultural operations, and point sources, may provide a more significant contribution to surface water contamination than previously recognised.

MATERIALS AND METHODS

German Study (The Dammbach Catchment)

This study was conducted as a joint agrochemical (Rhône-Poulenc Agro, AgrEvo and Stefes) and water industry (Gelsenwasser AG) initiative. The objective was to determine to what extent practical measures taken by farmers can significantly reduce the load of plant protection products in surface waters. The catchment consisted of 600 ha of which 320 ha were cereals. There were 30 farms involved between 20-40 hectares each, 20 sprayers, 4 of which were contract machines. The catchment is situated in the Stever region of Westfalia, Germany.

Following a year of monitoring to assess base levels of phenyl urea herbicides in surface waters, the issue of water contamination was outlined to the local farmers. Improvements were made to sprayers, including the fitting of filling devices, the use of anti-drift nozzles, and an additional clean water tank and brush for sprayer decontamination. Advice given was to follow good agricultural practice, to dilute spray tank residues and apply to a previously treated area where this could be done in accordance with label maximum application rates. In addition, it was recommended to fill and clean the sprayer away from water courses, which was best done in the field thus avoiding farm-yard pesticide runoff to the drains.

UK Study (Cherwell Catchment)

The first year of a two year study established in October 1998 had as an objective the quantification of the relative significance of point and diffuse sources of IPU contamination to surface water. The site was a small agricultural catchment situated at the headwater of the River Cherwell, (Northamptonshire, UK). It differs fundamentally from Dammbach by being occupied by only one farm. It comprises of 100 ha of which ~40% is winter cereals. The soil is a clay loam and tile and mole drained to ditch systems or to the river. The farmyard is covered with concrete and drains directly into the River Cherwell.

Automated water monitoring data systems were located at the catchment outlet and farm yard. The data provides a method for the quantification of IPU losses from farm operations. An assessment of the contribution from diffuse sources can be estimated by mass balance calculations - both as concentration and loading over time.

IPU was applied at a rate of 2.5 kg ai/ha^{-1} as normal farm practice to winter barley on 17th November 1998 by a self propelled sprayer. Tank filling was carried out on four occasions on the concrete yard. After the fourth tank mixing the yard was pressure washed with water.

To estimate the amount of pesticide adhering to the external surfaces of the sprayer during application, swabs were taken from representative areas, pre and post application, and following external washing. On completion of spraying the internal surface of the spray tank were rinsed twice and a sample of liquor taken from the sprayer sump.

RESULTS

German Study (The Dammbach Catchment)

With comparable amounts of IPU applied during the autumns of 1996/97 and 1997/98 season, the percent loading of IPU being found in the Dammbach leaving the catchment area in the autumn was reduced by 70% despite higher rainfall of the 1997/98 season.

Comparing the two years, the discharge of phenyl urea herbicides was reduced by 60 %.

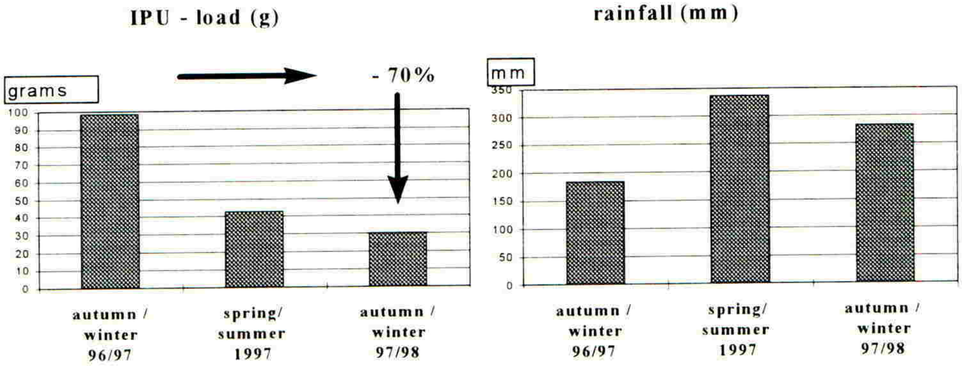


Figure 1.IPU-load and rainfall per season in the Dammbach area

UK Study (Cherwell Catchment)

IPU was not detected at any sampling points prior to application.

Despite working to a high professional standard, there were a number of operator activities that led to contamination of yard and hence the river (Table 1). A loading of 5 g of IPU was calculated to have left the yard within the first two months following application. It should be noted that even after six months small amounts of IPU (2.5 mg/Kg $^{-1}$) were recorded in the mud taken from the yard surface.

Table 1. Contribution of IPU contamination from yard activities (17/11/98 to 20/01/99)

Distribution	Total grams
Tank Rinsing (1 litre)	0.5
Spillage (tank mix)	1.2
Container Washings Spill	0.35
Tractor Washing	0.7
Tractor Wheels	0.4

DISCUSSION

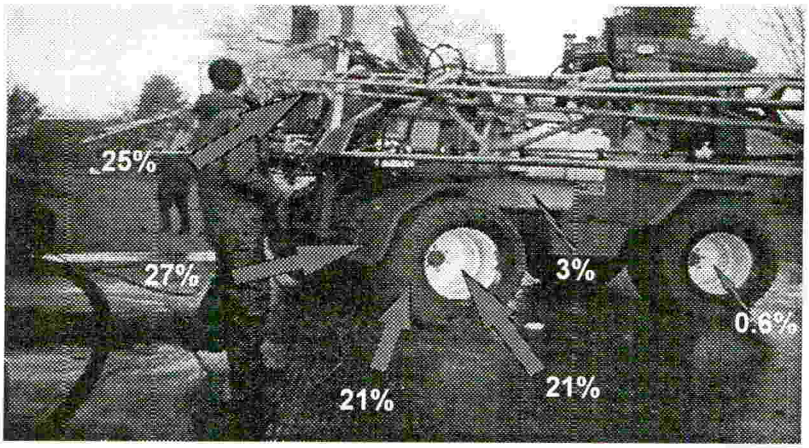
The studies indicate the importance of point sources as one of the major routes for surface water contamination. Dammbach demonstrates the importance of sprayer maintenance to prevent dripping nozzles and over-filling as well as the practice of working away from drains and streams and working off concrete. The Cherwell study highlights the importance of minute splashes of concentrate, discarded container foil seals, and sprayer contamination.

There was no spillage of product during the UK study, though "micro spills" were observed during loading, when small incidences of contamination to the yard area occurred from glugging and splashing. Taps and vehicle mudguards became smeared from gloves contaminated with concentrated product. Results indicate that at least 0.4 mg of IPU was distributed on the yard by these means.

The sprayer was well maintained, there were no leaking nozzles or pipe-work. However a small leakage of tank mix from a rear overflow pipe was noted on the first loading operation this contributed ~1.2 g of IPU to the yard surface.

Rinsed containers were returned upside down to their boxes, 0.35 g of IPU leaked from these containers. This is a common practice on many farms.

It was estimated that concentrated product adhering to a foil seal can contribute between 0.5 and 1.0g of active of a 50% formulation. Their contribution at Cherwell was not separated.



Picture 1. Areas of maximum sprayer contamination.

Excluding the contribution from mud, it was calculated that a total of circa 0.7 mg of IPU was available to be washed into the river as a result of tractor washing. This equates to 0.0007% of product applied. It should be noted that the sprayer had been pressure hosed and treated with a water repellent prior to arriving on site and it is not unreasonable to assume that this is the least contamination that may be expected. Other studies to maximise sprayer contamination indicate 1% of applied product adhering to the sprayer (Cooper & Taylor, 1998). A more likely contamination level may be 0.04% as recorded on a previous IPU study (Fogg, 1999).

Picture 1 visually demonstrates the major areas of sprayer contamination and where washing should be focused.

Mud samples taken from the rear tyres after spraying contained between 8 to 10 mg kg^{-1} of

IPU. It was estimated that approximately 40 kg of mud remained on the yard. The presence of this mud lodged in cracks and hollows of the concrete yard, may be the reason why contamination from the yard continued until monitoring ceased 6 months after application.

Some operators may discharge the final rinsate from the spray tank onto the farm-yard, with the assumption that following two rinses the sprayer is clean. However, the estimated 25 litres of rinsate remaining in the tank sump in this study, if discharged, would have contributed 12 g of IPU directly to the river.

Solutions to Yard Contamination

The EU Drinking Water Directive applies a stringent standard for pesticide levels in tap water of 0.1 ppb. It does not set a level for surface waters. This figure does little to highlight the importance of preventing small amounts of pesticide reaching water. Minute amounts mean little to the farmer who measures quantities in kilograms and hundreds of litres. Communicating the importance of small amounts of contamination to the farmer is essential to the stewardship of pesticides. The significance of these figures, can be conveyed by describing the water volumes required to dilute “micro-spills” of concentrate in order to meet the 0.1 ppb level, equating these to the length of a stream 1 meter wide by 30 centimetres deep.

In the UK study it was estimated that 5 g of IPU entered the river from farm yard operations, this would require a dilution of 50,000,000 litres of water which is sufficient to fill a stream 150 km in length. In practice pesticide, attenuation takes place which will reduce this figure.

There are four operational areas where the risk to surface water is high and for which solutions are possible:

1. machinery maintenance and cleaning
2. containers handling and design
3. yard operations
4. field operations

1. Machinery

a) Farmer based solutions.
Sprayers should be regularly serviced with nozzles replaced when worn. Pipe-work and joints should be sound. A leaking nozzle dripping every 20 seconds for ten minutes can pollute 13 km of stream.

b) Industry based solutions.
New sprayers should be fitted with external washing equipment, older machines can have such equipment retro-fitted. Induction bowls need to incorporate anti-glugging devices, and preparation areas on the sprayer are required that drain into the tank. Reduced sump sizes and accessible sump drains.

2. Containers

a.) Farmer based solutions.
Empty containers should be carefully handled and should be treated as full. the stored safely,

securely and undercover, prior to disposal. Foil-seals should be directly placed in empty boxes, as should upright containers once they are well rinsed with lids tightly replaced.

b.) Industry based solutions.
Containers should not incorporate removable foil seals. Bottles should be anti-glugging, easily rinsed and made of clear or opaque material to check cleanliness after rinsing.

3. Yard operations.

An area away from concrete that does not put streams or ditches at risk is most suitable. Washing the sprayer on the yard whether or not it drains to a ditch or stream, contravenes the Ground Water Directive. Sprayers should be regularly washed in the field of application, or on an Environment Agency approved area.

Deal will spillage immediately (no matter how small). Absorbent matting, granules are ideal to contain a spill. Have a spill contingency plan. A spill left to dry is as bad as hosing it down the drain.

4. Field operations.

Moving the spray preparation and sprayer washing to the field of application will reduce surface water contamination to a minimum, but ensure that streams and ditches are not put at a greater risk. Do not siphon water directly from streams or ditches. If possible, spray headlands last to reduce contaminated mud adhering to tyres. Cleaning the exterior of sprayer should focus on the key areas such as wheel hubs and centre/back of sprayer.

Yard contamination is controllable, but it is not an issue solely for the farming community. Close co-operation is required between Water and Crop Protection Industries, together with farmer organisations, machinery manufactures and regulatory authorities, to provide a full suite of solutions that can be adopted by all farms using crop protection products in the UK. Such co-operation will provide long-term solutions to this issue, more immediately the simple practices outlined can reduce peak levels of contamination in surface water.

CONCLUSION

It is concluded that farm yard operations (point-source) have a significant impact on the level of pesticides being found in surface water. It is further concluded that a stewardship programme based on simple practical solutions will reduce yard contamination and in turn reduce peak levels of contamination in surface water. Such a reduction is required if water companies are to continue to meet the stringent standard for pesticide levels set out in the Drinking Water Directive.

REFERENCES

Cooper S E; Taylor W A(1998) Some factors that may influence rate of accumulation and final quantity of pesticide deposits on external surfaces of arable sprayers. *1998 BCPC Symposium proceedings.*

Fogg P (1999). Pesticide Residues From Spray Equipment 1999 *(unpublished)*

Solid urban waste residue amendment as affecting simazine and 2,4-D leaching in soils

L Cox, J Cornejo, R Celis, M C Hermosín

Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, PO Box 1052, 41080 Sevilla, Spain

ABSTRACT

We have studied the sorption and leaching of simazine and 2,4-D on a Typic Rhodoxeralf soil (0.66 % organic C, 75 % sand, 16 % clay) and in this soil amended with a composted solid urban waste (SUW) residue (28 % organic matter). Sorption was studied following a batch equilibration technique and sorption isotherms fitted to the Freundlich equation. Leaching was studied in handpacked soil columns under fluctuating saturated/unsaturated flow conditions. Simazine sorption coefficients (Kf) increased with SUW amendment from 0.44 (R^2= 0.95) to 0.64 (R^2= 0.96). No sorption of 2,4-D was measured on the unamended soil whereas Kf for the SUW soil was 0.60 (R^2= 1.00). The maximum concentration of herbicide detected in leachates was three times higher in the original soil than in the SUW soil in the case of simazine and four times higher in the case of 2,4-D. Simazine recoveries in leachates were 52 % of applied in the original soil and 32 % in the SUW soil. 2,4-D recovery in leachates was dramatically reduced with the SUW amendment from 100 to 25 % of applied. Results indicate that SUW amendment reduces herbicide leaching by promoting irreversible sorption and probably by enhancing degradation.

INTRODUCTION

Soil organic matter is the primary sorbent for pesticides and sorption is one of the main processes reducing the mobility of these chemicals in the soil (Chiou, 1989). Thus, organic amendments can be used to modify surfaces of soils and sub-surface materials to promote adsorption and reduce pesticide contamination of ground water (Zsolnay, 1992; Guo *et al.*, 1991; Arrienzo *et al.*, 1994; Barriuso *et al.*, 1996; Cox *et al.*, 1997). On the other hand, the addition of organic amendments can also modify soil chemical and biological conditions, which can affect degradation and in some cases accelerate this process (Top *et al.*, 1996; Cox *et al.*, 1997a; Gan *et al.*, 1998) reducing the total amount of chemical potentially available for leaching. When the amendment is a residue such as urban waste, land application is also an alternative way of disposal.

The aim of this study was to assess the influence of a solid urban waste residue (SUW) amendment on sorption and degradation of the herbicides simazine (6-chloro-*N,N'*-diethyl-=1,3,5-triazine-2,4-diamine) and 2,4-D ((2,4-dichlorophenoxy)acetic acid), which would determine the amount of chemical available for leaching through the soil profile and the risk of ground water contamination.

MATERIALS

Pure analytical simazine (chemical purity > 99 %) was supplied by Riedel de Häen AG. Pure a analytical 2,4-D (chemical purity > 99 %) was supplied by Sigma.

The 50 cm of a Typic Rhodoxeralf soil was sampled, air dried and sieved to pass a 2 mm mesh. In the laboratory, a solid urban waste compost (SUW) was added to the original soil at 10 % (w/w) rate. Physicochemical properties of the original (unamended) soil and the SUW amended soil were determined and are given in Table 1 together with some physicochemical properties of the SUW amendment.

Table 1. Selected physicochemical properties of the soils and SUW amendment.

	pH	Organic matter %	Sand %	Clay %
Unamended	7.1	1.14	75.4	16.4
SUW soil	7.5	3.02	Id	id
SUW	7.8	31.5	--	--

METHODS

Sorption-Desorption Studies

Sorption studies were performed using batch equilibration procedure. Duplicate samples of 5 g of soil were treated with 10 mL of simazine solutions of initial concentrations (Ci) ranging from 0.5 to 10 µM or Ci ranging from 5 to 100 µM of 2,4-D. The suspensions were shaken at 20 ± 2°C for 24 h and then centrifuged at 31000 g at the same temperature. Equilibrium concentrations (Ce) in the supernatants were determined by HPLC under the following conditions: Nova-Pack column of 150 mm length x 3.9 mm i.d.; column packing, C18; flow rate, 1 ml/min; eluent system, 70:30 water-acetonitrile mixture for simazine and a 45:55 methanol-H_3PO_4 (pH 2) mixture for 2,4-D and UV detection at 230 nm.. Differences between Ci and Ce were assumed to be the amounts adsorbed (Cs). Sorption isotherms were fitted to Freundlich equation (logCs=logKf + nflogCe) and Kf (sorption capacity) and nf (sorption intensity or linearity) calculated.

Desorption experiments were conducted inmediately after the sorption experiment using soils from the 10 µM (simazine) or 100 µM (2,4-D) initial solution concentrations. After centrifugation in the sorption experiment and 5 mL removed for analysis, 5 mL of 0.01 M $CaCl_2$ without chemical were added to the soil. Soils were resuspended and shaken for 24 h. Soil suspensions were centrifuged and 5 mL of supernatant removed for analysis. This desorption cycle was repeated three times for each sample.

Incubation Studies

Duplicate amounts (400 g) of unamended and SUW soil were treated with a solution of simazine or 2,4-D to give a concentration of 5 mg/kg dry soil or 2.3 mg/kg, respectively. Moisture content was adjusted to -0.33 kPa. Soil samples were incubated at 20°C for 28 days. Moisture contents were mantained at a constant level throughout the experiment by adding distilled water as necessary. Soils were sampled periodically and herbicides extracted by shaking for 24 h 10 g of soil with 20 mL of methanol in the case of simazine and with 20 mL of a 45:55 methanol-H_3PO_4 (pH 2) mixture. Soil extracts were analysed by hplc.

Leaching Experiments

Leaching was studied in handpacked soil columns (30 cm long x 5 cm inner diameter). Before the application of the herbicides, columns were conditioned with 250 mL of water and then allowed to drain for 24 hours. In this way pore volumes (V/V_o) were calculated. The amount of simazine or 2,4-D corresponding to an application rate in soils of 5 kg/ha or 2.3 kg/ha was added to the top of the columns. Twenty four hours after herbicide application, columns were leached with water which was applied at a rate of 25 mL/day. Leachates were collected daily, filtered and analyzed directly by HPLC for their herbicide content.

RESULTS AND DISCUSSION

Sorption-Desorption Studies

Simazine sorption-desorption isotherms in the unamended soil and SUW soil are given in Figure 1a . Sorption isotherm in the original soil is L-type (Giles *et al.*,1960) which indicate a decrease of site availability as the solution concentration increases, whereas in the SUW soil sorption isotherm is C-type, indicating a constant partition solid/solution often showed in interactions between non-ionic compounds and organic surfaces. Sorption-desorption isotherms were fitted to Freundlich equation and sorption coefficients Kf and nf calculated (Table 2). Kf and nf indicate sorption capacity and sorption intensity, respectively. Both sorption coefficients increased with the organic amendment, due to the increase in organic matter content of the soil. Desorption isotherms indicate that simazine sorption in the unamended soil is reversible, since sorption and desorption branches (Fig.1a) were almost identical. Desorption from the SUW soil was not reversible, as shown in Figure 1a and confirmed by a lower value for nf_{des} than for nf_{ads} (Table 2). This irreversibility can be attributed to simazine bound to the organic matter, which is increased with the amendment, since hydrophobic molecules such as simazine are more tightly bound to organic components than to inorganic soil components (Xing *et al.*, 1997).

2,4-D did not sorb on the unamended soil whereas sorption isotherm on SUW soil is given in Figure 1b. Sorption coefficient Kf is similar to simazine in SUW soil, although the nf value is much lower in the case of 2,4-D than for simazine. Desorption is irreversible, as shown also in Figure 1 and indicated by lower nf_{des} than nf_{ads} in Table 2. This difference between sorption and desorption intensity (nf_{ads} and nf_{des}) can also be attributed to degradation during desorption experiment or to slow desorption equilibrium (Koskinen & Cheng, 1983)

Table 2. Simazine and 2,4-D sorption-desorption coefficients calculated fitting isotherms to Freundlich equation.

Soil	Simazine					2,4-D				
	Kf	nf_{ads}	R^2_{ads}	nf_{des}	R^2_{des}	Kf	nf_{ads}	R^2_{ads}	nf_{des}	R^2_{des}
Original	0.44	0.83	0.95	0.95	0.92	--	--	--	--	--
SUW	0.64	1.25	0.96	0.97	1.00	0.60	0.69	1.00	0.32	0.97

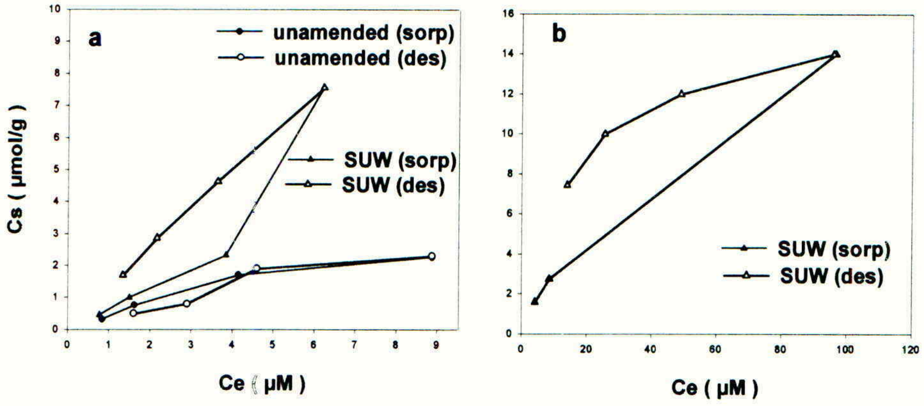


Figure 1. Simazine (a) and 2,4-D (b) sorption-desorption isotherms.

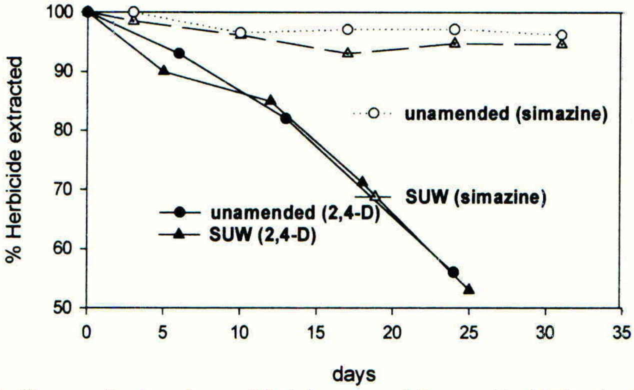


Figure 2. Changes in simazine and 2,4-d extracted from soil with incubation time.

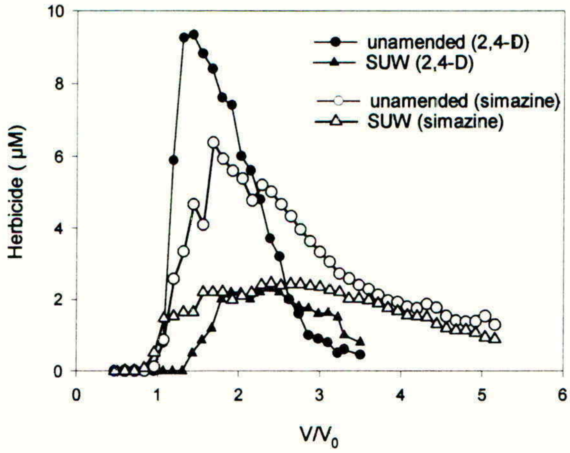


Figure 3. Simazine and 2,4-D BTCs in unamended and SUW soil.

Incubation Studies

Figure 2 shows the changes in total simazine and 2,4-D recovered from the soil with incubation time. Nearly 100 % of the applied simazine is recovered after 28 days in both unamended and SUW soil (Fig.2). 2,4-D showed shorter persistence than simazine (Wauchope *et al.*, 1992). The half lives, calculated after fitting 2,4-D dissipation curves to first order kinetics, were 28 d for the unamended soil (R^2= 0.95) and 29 d for SUW soil (R^2= 0.93). The increase in OC content of the soil with the amendment (Table 1) that, in general, has been shown to promote biodegradation (Guo *et al.*, 1993; Cox *et al.*, 1997; Gan *et al.*, 1998) does not seem to affect either 2,4-D or simazine dissipation in soil under the conditions studied.

Leaching experiments

Simazine and 2,4-D breakthrough curves (BTCs) in the unamended soil and SUW soil are shown in Figure 3. Variations in measurements made on leachate samples from replicate columns was generally within 5 % of the appropriate mean value for the three soils.

Simazine initial breakthrough occurs at the same time in the two soils. BTC in SUW soil is very flat, without a clear position of the maximum concentration peak. Simazine concentrations in leachates from SUW-soil are much lower than those from the unamended soil, which can be attributed the sorption process favoured in SUW soil. The asymmetry and extensive tailing of the BTC is indicative of non-equilibrium sorption during leaching (Beck *et al.*, 1993; Garmendier *et al.*, 1993), which agrees with the lower reversibility of simazine sorption in SUW soil. After the leaching experiment, 52 % of the of initially applied simazine was recovered in leachates of the unamended soil whereas only 32 % of initially applied simazine was recovered from SUW soil.

2,4-D initial breakthrough in the unamended soil occurs at the same time as simazine, although the maximum concentration appears earlier, in agreement with no 2,4-D sorption in the unamended soil. 2,4-D initial breakthrough occurs later in the SUW soil than in the unamended soil and the position of the maximum is shifted to higher pore volumes. Both facts are due to sorption (Garmendinger *et al.*, 1993), which increases with SUW amendment to a higher extend when compared with simazine. The differences between BTCs in the unamended soil and SUW soil were much larger for 2,4-D than for simazine. The amount of 2,4-D leached from the unamended soil was nearly 100 % of applied, whereas only 25 % leached from SUW soil. The retardation of the movement of 2,4-D due to sorption process favours degradation by increasing its residence time (Cox *et al.*, 1997) in the amended soil, although this effect is not shown in the incubation experiments. The saturated/unsaturated conditions seem to favour degradation of 2,4-D in the SUW amended soil.

CONCLUSIONS

Results from this study indicate that soil organic amendment with solid urban waste (SUW) increases the amount of simazine and 2,4-D sorbed and the irreversibility of the adsorption. This increase in sorption decreases the amount of herbicide in soil solution available for leaching and increases their residence time in the soil column favouring degradation processes. Both sorption and degradation account for the low recoveries of simazine and 2,4-D in soil column experiments.

ACKNOWLEDGEMENTS

This work is supported by Junta de Andalucía through Research Group RNM 124 and CICYT through Project AMB96-0445-CO2-O2.

REFERENCES

Arienzo M; Sánchez-Camazano M; Sánchez-Martín M J; Crisanto T (1994) Mobility of diazinon in soils modified by addition of organic matter. pp. 134-137. In: A. Copin *et al.* (eds.), *Environmental Behaviour of Pesticides and Regulatory Aspects*, European Study Service, Belgium.

Barriuso E; Houot S; Serra-Wittling C (1996). Influence of compost addition to soil on the behaviour of herbicides. *Pesticide Science* **49,** 65-75.

Beck A J; Jonston A E; Jones K C (1993). Movement of nonionic organic chemicals in agricultural soils. *Critical Reviews in Environmental Science & Technology* **23,** 219-248.

Chiou C T (1989). Theoretical considerations of the partition uptake of nonionic organic componds by soil organic matter. pp. 1-29. In: *Reactions and Movement of Organic Chemicals in Soils.* Soil Science Society of America Special Publication N 22.

Cox L; Celis R; Hermosín M C; Becker A; Cornejo, J (1997). Porosity and herbicide leaching in soils amended with olive-mill waste water. *Agriculture, Ecosystems & Environment* **65,** 151-161.

Gan J S; Yates R; Crowley D; Becker J O (1998). Acceleration of 1,3-Dichloropropene degradation by organic amendments and potential application for emissions reduction. *Journal Environmental Quality* **27,** 408-414.

Garmendier A P; Dowling K C; Lemley, A T (1993) Miscible displacement and theoretical techniques for simultaneous study of pesticide sorption and degradation during transport. pp 115-123. In: *Sorption and degradation of pesticides and organic chemicals in soil.* Soil Science Society of America Special publication No. 32. Madison. WI.

Giles, C H; McEwan, S N; Nakhwa S N; Smith D (1960). Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms and its use in diagnosis of adsorption mechanisms and in measurement of specific surface area in solids. *Journal Chemical Society,* 3973-3993.

Guo L; Bicki T J; Hinesly T D (1991) Effect of carbon-rich waste materials on movement and sorption of atrazine in a sandy, coarse textured soil. *Environmental Toxicology Chemistry* **10,** 1273-1282.

Koskinen W C; Cheng H H (1983) Effects of experimental variables on 2,45-T adsorption-desorption in soil . *ournal. Environmental Quality* **12,** 325-330.

Wauchope R D; Butler T M; Hornsby A G; Augustijn-Beckers PWM; Burt J P (1992). The DCS/ARS/CES pesticide properties database for environmental decision making. *Reviews of Environmental Contamination and Toxicology* **123,** 1-164.

Worthing, C R; Hance, R J (1991) *The Pesticide Manual.* British Crop Protection Council, Surrey, U.K.

Xing B; Pignatello J J (1997). Dual-Mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter. *Environmental. Science Technology.* **31,** 792-799.

Zsolnay, A (1992) Effect of an organic fertilizer on the transport of the herbicide atrazine in soil. *Chemosphere* **24,** 663-669.

Influence of soil pH-sorption interactions on imazamox carryover

G A Bresnahan, A G Dexter
Dept of Plant Sciences, North Dakota State University, Loftsgard Hall, Fargo, ND 58105, USA

W C Koskinen
USDA-ARS, 1991 Upper Buford Circle, Rm 439, St. Paul, MN 55108, USA

W E Lueschen
Dept of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

ABSTRACT

Long-term carryover of some imidizolinone herbicides has been observed in soil that is below pH 6.5, resulting in significant sugarbeet damage. In a field study in Southwest Minnesota, imazamox concentration decreased rapidly in field soil, regardless of pH. Despite similar amounts of imazamox remaining in aged soils at different pH levels, bioavailability differed with pH, which can be explained by sorption-desorption. At low pH more imazamox was sorbed than at high pH, but it readily desorbed. At high pH less imazamox was sorbed initially, but it did not readily desorb. Thus, after three months, the remaining imazamox in low pH soil was desorbable and bioavailable, resulting in injury to canola and sugarbeet. Adding lime to aged, low pH soil, released bound imazamox residues, which would then be degraded and result in less carryover.

INTRODUCTION

One of the most important processes affecting the fate of herbicides in the field is sorption. Sorption controls the availability of the herbicide to the target plant, soil microorganisms, and for movement of the chemical through the soil profile. Soil organic carbon (OC) content is the most important soil property affecting sorption of nonpolar, nonionizable organic chemicals (Koskinen & Harper, 1990). For polar, ionizable chemicals, such as weak acids like imazamox, the most important factor affecting sorption is pH but, at lower pH levels, sorption of ionizable chemicals can also be influenced by soil OC content (Oliveira *et al.*, 1999). Sorption of pesticides has also been shown to be affected by aging in soil (Cox *et.al.*, 1998). The increase in sorption with time would result in decreased availability for degradation and movement.

The objective of the current research was to determine the influence of soil pH/sorption interactions as a function of aging on imazamox soil carryover as indicated by sugarbeet injury. A secondary objective was to determine if increasing soil pH with lime from sugar processing of sugarbeet would decrease sorption of aged imazamox residues and subsequent potential carryover.

METHODS AND MATERIALS

Field Experiment

Two fields located in Southwest Minnesota were used in the experiment. Field One soil was Ves loam soil and Field Two soil was a combination of Ves loam soil and Webster loam soil. Soil OC content ranged from 2.1 to 2.3%. In 1996, plots were treated with lime at 8 and 26 t ha^{-1} with spent lime (86% calcium carbonate equivalent) from nearby sugarbeet processing plant, or left unlimed. Field One was seeded with 'Pioneer 9137' soybean (*Glycine max* L). Field Two was seeded with 'Land O Lake 0946' soybean. Imazamox was applied at 0.035 kg ha^{-1} at the 3- to 4-trifoliolate leaf stage. Samples were collected from all plots three times during the growing season. Soil samples were frozen at -10°C until pH and imazamox residues were determined. In 1997, Field One was seeded with 'Viking' sugarbeet (*Beta vulgaris* L.). Sugarbeet was hand harvested from one row, 3 m in length. Sugarbeet was analyzed for yield, % sucrose and extractable sucrose. Field Two was seeded to 'Pioneer 2375' wheat (*Triticum aestivum* L.). In 1998, Field Two was seeded with "American Crystal 309" sugarbeet. Yield data was obtained by hand harvesting two rows, 3 m in length. The sugarbeet crop was analyzed for yield, % sucrose and extractable sucrose.

Soil Analyses

Imazamox residue analyses were conducted using method M 2726, provided by American Cyanamid Company. Imazamox was extracted with NaOH, cleaned up by solid phase extraction and quantified by HPLC-UV detector at 254 nm. A C8 reversed-phase Zorbax column with a mobile phase of acetonitrile:water:formic acid (32:66:2) at a flow rate of 1.00 ml min^{-1} was used. Sample injection volume was 200 μL.

Sorption-Desorption

Ves loam soil samples were used for sorption-desorption and aged residue studies. The samples were from a previous liming experiment which produced variable pH plots within a single field. The pH of the soil samples ranged from 5.4 to 7.7, with the OC content ranging from 2.26 to 3.31%. Sorption was characterized using the batch equilibration technique, using soil freshly spiked with imazamox at four concentration levels, 1.6 to 48 mg kg^{-1}, and contained 126 to 148 Bq ^{14}C-labeled imazamox (American Cyanamid, Princeton, N.J., specific activity=116.6 μCi mg^{-1}). Desorption experiments were conducted immediately after the sorption experiment using the soils from all four initial concentrations.

Greenhouse Experiment

Pre-germinated imazethapyr-resistant and -susceptible canola were seeded at 11 kg ha^{-1} into soil samples taken from limed and unlimed plots. Soil from the three field sampling times, immediately, 9, and 18 weeks after imazethapyr application was used. Plants were grown in the greenhouse at 25 ± 2°C with supplemental lighting from Phillips 40 W Agri-grow lights. Canola growth was measured during a 3-wk period.

RESULTS

Field Results

The pH of the field plot soil increased from 5.7 to 6.7 in the field plots limed at 8 t ha^{-1}, and to 7.7 in soil limed at 26 t ha^{-1}. Imazamox concentration decreased rapidly in the surface (15 cm of soil) during the 1996 growing-season, 82% dissipated within 3 mo (Table 1). Soil pH had no effect on the initial dissipation, approximately 16 µg kg^{-1} remained in all three soils with different pHs 3 mo after application.

Table 1. Imazamox field dissipation as a function of pH.

Lime (t ha^{-1})	pH	Time after application (wks)	Concentration (ppb)
0	5.7	0	89.5 ± 16.3[a]
		6	53.7 ± 4.5
		12	17.1 ± 1.1
8	6.8	0	91.8 ± 3.9
		6	47.5 ± 4.2
		12	16.8 ± 0.6
26	7.7	0	89.0 ± 8.4
		6	42.0 ± 4.8
		12	14.1 ± 15.1

[a]values ± standard error

In the imazamox-treated plots the year following liming and imazamox application (1997), imazamox carryover was evident at all three pH levels; imazamox residue was sufficient to cause some injury to sugarbeet. Although similar amounts of imazamox remained at the end of the growing season, it appears pH affected its bioavailability as indicated by effects on canola growth and sugar beet injury. At all pH levels and at all sampling times, imazamox affected growth of susceptible canola but did not affect resistant canola (Table 2). Although the same amount of imazamox was present for each pH soil at each sampling time (discussed above), the imazethapyr present was more bioavailable at pH <6 than at pH >6.

Table 2. Imazamox and pH effects on canola in the greenhouse.

		Canola height (cm)	
Lime (t ha^{-1})	pH	Resistant canola	Susceptible canola
0	5.7	4.9	0.7
8	6.8	6.4	3.3
26	7.7	6.3	3.8
LSD (0.05)		0.8	0.9

Plots limed at 26 t ha^{-1} and treated with imazamox produced 16.1 t ha^{-1} greater sugarbeet root yield and 2353 kg ha^{-1} more extractable sucrose than unlimed plots treated with imazamox (Table 3). Imazamox-treated plots limed at 8 t ha^{-1} produced 8 t ha^{-1} greater sugarbeet root yield and 1345 kg ha^{-1} more extractable sucrose than unlimed plots treated with imazamox. At all three pH levels imazamox-treated plots had lower root yields and extractable sucrose than no-herbicide plots.

Table 3. Effect of imazamox and pH on root yield, extractable sucrose, and % sucrose.

Year	Treatment	Applied lime (t ha^{-1})	Soil pH	Root yield (t ha^{-1})	Extractable sucrose (Kg ha^{-1})	Sucrose %
		8	6.8	50.9	7734	14.6
		26	7.7	48.0	6613	14.7
	Imazamox	0	5.7	29.0	3475	13.4
		8	6.8	37.0	4820	14.1
		26	7.7	45.1	5828	14.3
	LSD (0.05)			4.3	1110	0.6
1998	None	0	5.8	48.0	7955	18.0
		8	6.6	51.8	8861	18.5
		26	7.5	65.7	10749	17.8
	Imazamox	0	5.8	51.1	8451	16.1
		8	6.6	51.5	8620	17.9
		26	7.5	56.3	9007	17.8
	LSD (0.05)			10.4	1832	3.2

In 1998 (2 yr after liming and imazamox application), sugarbeet root yields in the treated plots were not significantly different from the untreated plots, regardless of pH. Plots not treated with herbicide had greater sugarbeet root yields and extractable sucrose when lime was used to increase soil pH (Table 3).

Sorption-desorption

Imazamox sorption isotherms fit the Freundlich equation ($r^2 > 0.96$) and in most cases were linear ($1/n_f \geq 0.95$) at soil pH levels from 5.4 to 7.7, indicating that sorption is not dependent on concentration (Table 4). The fact that Freundlich isotherm slopes, $1/n_f$, were not significantly different for all soils (except for pH 7.4 soils) allowed comparison of the Freundlich K_f constants as a measure of the extent of sorption for the different soils in the range of concentrations used. Less herbicide was sorbed onto soils with high pH (7.7) than on low pH soils, consistent with previous research on related chemicals (Che *et al.*, 1992; Bresnahan *et al.*, 1999). Soil with a pH = 7.7 had K_f values < 0.3, whereas for pH 6.2 soil, K_f was 0.8, while pH 5.4 soil had a K_f value of 1.3.

Table 4. Imazamox sorption-desorption as a function of pH.

pH	K_f ($mg^{(1-1/n)}$ kg^{-1} $L^{1/n}$)	$1/n_f$	$1/n_{fd}$[a]	K_{foc} ($mg^{(1-1/n)}$ kg^{-1} $L^{1/n}$)
7.7	0.26 ± 0.08[b]	1.01 ± 0.13	0.17 ± 0.03	11
7.4	0.82 ± 0.15	0.72 ± 0.02	0.05 ± 0.05	36
6.2	0.81 ± 0.23	0.97 ± 0.04	0.31 ± 0.18	35
5.7	1.19 ± 0.07	0.95 ± 0.02	0.41 ± 0.09	52
5.4	1.30 ± 0.20	0.98 ± 0.02	0.40 ± 0.02	57

[a] $1/n_{fd}$ = the mean desorption isotherm slopes for 0.024 μ ml^{-1} initial concentration

[b] values ± standard error

Desorption isotherms fit the Freundlich equation ($r^2 > 0.90$) and hysteresis was observed during desorption from all pH soils ($1/n_{fd} < 1/n_f$) (Table 4). Hysteresis was previously observed in various soils for a related chemical (Gan *et al.* 1994; Bresnahan *et al*, 1999). Greater desorption hysteresis was observed at higher soil pH levels, than at low soil pH. For instance, at pH of 7.4, $1/n_{fd} < 0.2$, while at pH < 6.2, $1/n_{fd}$ was > 0.3. This indicates that although less imazamox is sorbed at a high pH, what is sorbed is more resistant to being desorbed from soil, while at low pH the imazamox is more readily desorbed.

CONCLUSIONS

Although soil pH did not affect imazamox persistence in both field and laboratory experiments, pH appeared to affect bioavailability, resulting in carryover effects on sugarbeet. For instance, although 82 % of the applied imazamox had dissipated 3 mo after application, the remaining residues were more bioavailable at low pH as indicated by greater effects on susceptible canola and sugarbeet. Two years after imazamox application no residues were present to affect sugarbeet root yield. The increase in sorption with aging appeared to be in part, a function of the rate of formation of imazamox cation. In low pH soil, greater amounts of cation are formed and sorbed by the soil colloid than in high pH soil during the aging period, a process that is reversible. Raising soil pH with lime would reduce sugarbeet injury from carryover of imazamox, but would not totally prevent sugarbeet injury one year after application. Results of these experiments also suggest that raising soil pH with lime in soils with a low pH will increase the sugarbeet yield potential of the soils even in the absence of herbicide residue.

ACKNOWLEDGMENT

This material is based upon work supported by the Cooperative States Research Service, United States Department of Agriculture, under Agreement No. 93-34297-8354. We thank American Cyanamid for kindly supplying the 14_{C}-labeled imazamox.

REFERENCES

Bresnahan, G A; Koskinen W C; Dexter A G; Lueschen W E (1999). Influence of soil pH-sorption interactions on imazethapyr carryover. *Journal of Agricultural and Food Chemistry* (Submitted).

Che M; Loux M M; Traina S J; Logan T J (1992). Effect of pH on sorption and desorption of imazaquin and imazethapyr on clays and humic acid. *Journal Environmental Quality* **21**, 698-703.

Cox, L; Koskinen W C; Yen P Y (1998). Changes in sorption of imidacloprid with incubation time. *Soil Science Society of America Journal* **62**, 342-347.

Gan J; Weimer M R; Koskinen W C; Buhler D D; Wyse, D L; Becker, R L (1994). Sorption and desorption of imazethapyr and 5-hydroxyimazethapyr in Minnesota soils. *Weed Science* **42**, 92-97.

Koskinen, W C; Harper, S S (1990). The retention process: Mechanisms. In *Pesticides in the soil environment: Processes, impacts and modeling.* Cheng H H, Ed.; SSSA Book Ser. No. 2. pp 51-77. SSSA, Madison, WI.

Oliveira, R S Jr; Koskinen, W C; Ferreira, F A; Khakural, B R; Mulla, D J; Robert, P C (1999). Spatial variability of sorption/desorption of imazethapyr. *Weed Science* **47**, 243-248.

Organoclays and organohydrotalcites as sorbents for polar pesticides

R Celis, M C Hermosín, J Cornejo
Instituto de Recursos Naturales y Agrobiol., CSIC, PO Box 1052, 41080 Sevilla, Spain

W C Koskinen
USDA-ARS, 1991 Upper Buford Circle # 439, St. Paul, MN 55108, USA

ABSTRACT

The sorption and desorption of the anionic herbicide imazamox ($pK_{a1} \approx 3.9$, $pK_{a2} \approx 2.0$) by organoclays (alkylammonium-exchanged montmorillonite) and organohydrotalcites (alkylsulfate-exchanged hydrotalcite) was compared with that of the neutral fungicide triadimefon, to test the potential use of these sorbents for decontamination purposes and slow release formulations as related to the charge characteristics of the pesticide. In general, interlayered organoclays and organohydrotalcites were much more sorptive for imazamox and triadimefon than the corresponding natural or inorganic minerals. Depending on the sorbent, triadimefon sorption on organoclays and organohydrotalcites was 5 to 200 times greater than that observed for imazamox anion. In contrast, imazamox sorption to organoclays was irreversible, whereas triadimefon sorption by the organoclays and organohydrotalcites was moderate to low. Imazamox sorption, as well as the reversibility of the sorption-desorption process, increased with decreasing pH, due to protonation and further sorption of molecular imazamox species. The results of this work indicate that the diverse sorbents assayed may find application as filters in water decontamination for imazamox anion and as filters and supports for slow release formulations in the case of molecular imazamox and triadimefon.

INTRODUCTION

The need to protect and restore contaminated soils and aquifers is stimulating research to look for suitable materials to be used as pollutant sorbents, barriers and stabilisers (Jaynes & Boyd, 1991). Diverse materials are also being proposed for controlled release formulations to minimise the impact of pesticides in the environment (Darvari & Hasirci, 1996).

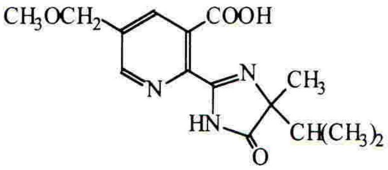
Natural clays and synthetic hydrotalcites are very good potential sorbents due to their layered structure. Because of the hydrophilic character of their surfaces, however, the sorption capacity of clays and hydrotalcites for organic molecules, such as pesticides, is usually low. Replacement of natural exchange ions with large organic ions through ion-exchange reactions has been shown to yield organoclays and organohydrotalcites with organophilic properties. Therefore, these materials have been proposed as sorbents for pesticides, especially those weakly retained by most soils and sediments, such as anionic or highly polar pesticides (Hermosin & Cornejo 1992, Zhao *et al.*, 1996).

In the present paper, the sorption and desorption of the anionic herbicide imazamox (2-[4,5-dihydro-4-methyl-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridine-carboxylic acid) by a series of organoclays (alkylammonium-exchanged montmorillonite) and organohydrotalcites (alkylsulfate-exchanged hydrotalcite) has been investigated and compared with that of the neutral fungicide triadimefon ([1-(chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone]), to test the potential use of these sorbents for decontamination purposes and slow release formulations as related to the charge characteristics of the pesticide. The effect of pH on sorption was also investigated.

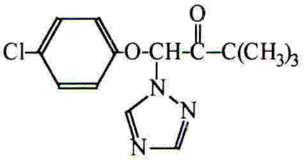
MATERIALS AND METHODS

Chemicals

Pure analytical imazamox (chemical purity > 99%) and radiochemical material (radiochemical purity > 98%) were supplied by American Cyanamid Company (Princeton, NJ). Pure analytical triadimefon (chemical purity > 99%) was purchased from Chem Service (West Chester, PA). The radiochemical triadimefon was donated by Mobay Chemical Corp. (now Bayer Corp., Stilwell, KS). It was purified by HPLC, and final radiochemical purity was > 98%. The chemical structures of triadimefon and imazamox are shown below.



Imazamox



Triadimefon

Sorbents

Table 1 summarises the characteristics of the different sorbents used. The main difference between SW and SA montmorillonites, supplied by the Clay Minerals Society, is the density of layer charge in the octahedral layer resulting in cation exchange capacities (CEC) of 76 $cmol_c$ kg^{-1} for SW and 120 $cmol_c$ kg^{-1} for SA.

Table 1. Characteristics of the sorbents[†].

Sorbent	Mineral phase	Interlamellar organic ion	OC	OISt	Basal spacing 25°C	Basal spacing 110°C
			%	%	nm	nm
SW	SWy2	-	-	0	1.26	0.98
SA	SAz1	-	-	0	1.52	1.49
HT	Hydrotalcite	-	-	-	0.76	0.76
SW-ODA_{50}	SWy2	Octadecylammonium	9.9	60	1.73	1.60
SW-ODA_{100}	SWy2	Octadecylammonium	14.8	90	1.72	1.76
SW-$HDTMA_{100}$	SWy2	Hexadecyltrimethylammonium	14.7	85	1.81	1.80
SA-ODA_{100}	SAz1	Octadecylammonium	25.5	98	3.25	2.25
SA-$HDTMA_{100}$	SAz1	Hexadecyltrimethylammonium	22.8	84	2.42	2.12
HTDDS	HT	Dodecylsulfate	26.0	91	2.67	2.67
HTDBS	HT	Dodecylbenzenesulfonate	28.0	73	3.12	3.12

[†] OC: organic carbon, OISt: fraction of the ion exchange capacity saturated by organic ion.

Two different organic cations (octadecylammonium, ODA, and hexadecyltrimethylammonium, HDTMA) were used in the synthesis of the organoclays. For the synthesis, 10 g of montmorillonite were treated with an amount of ethanol:water (50:50) solution containing the amount of alkylammonium chloride equivalent to 50% or 100% of the CEC of the clay. The suspensions were shaken at 20 ± 2°C for 24 h, centrifuged (10000 rpm, 10 min), washed with distilled water until Cl-free, then freeze-dried.

The hydrotalcite, HT [$(Mg_3Al(OH)_8)_2 \cdot CO_3 \cdot 4H_2O$], was prepared by the coprecipitation method described by Reichle (1986). An aliquot of the hydrotalcite was calcined at 500°C to obtain aluminium/magnesium mixed oxide, HT500. The organohydrotalcites were prepared by the reconstruction method, suspending 1 g of HT500 in 17 mM sodium dodecylsulfate (DDS) or sodium dodecylbenzenesulfonate (DBS). The suspensions were shaken for 24 h, thoroughly washed, then air-dried.

Sorption-desorption experiments

Imazamox and triadimefon sorption isotherms on the different sorbents were obtained in duplicate by the batch equilibration technique using 35 mL-glass centrifuge tubes with Teflon-lined caps. Initial pesticide solutions were prepared in 0.01 M $CaCl_2$ at concentrations ranging from 0.3 to 8.0 mg L^{-1}. Radiolabeled pesticide was added to nonradioactive solutions to give a final solution radioactivity of ~70 Bq mL^{-1}. Duplicate 10 mg (triadimefon) or 20 mg (imazamox) sorbent samples were equilibrated with 10 mL of pesticide initial solution by shaking mechanically at 20 ± 2°C for 24 h. After equilibration, the suspensions were centrifuged at 3000 rpm for 30 min, and the radioactivity of the supernatant determined by liquid scintillation counting using a 1500 Packard Instrument liquid scintillation analyser. The amount of pesticide in solution was calculated from the specific activity of the initial solutions. The effect of pH on sorption was determined at single pesticide concentration of 3 mg L^{-1} by adjusting the pH of the initial pesticide solution between 2.5 and 4.5 using HCl.

Desorption was measured immediately after sorption from the highest equilibrium concentration point of the sorption isotherms. The 5 mL of supernatant removed for the sorption analysis were replaced with 5 mL of 0.01 M $CaCl_2$. After shaking at 20 ± 2°C for 24 h, the suspensions were centrifuged and 5 mL supernatant removed for analysis. This desorption cycle was repeated four times. Desorption experiments were performed in triplicate.

Sorption and desorption isotherms were fit to Freundlich equation: $\log C_s = \log K_f + nf \log C_e$, where C_s (mg kg^{-1}) is the amount of pesticide sorbed at the equilibrium concentration Ce (mg L^{-1}), and K_f and nf are the Freundlich constants. Hysteresis coefficients, H, were calculated according to $H = (nf_{des})/(nf_{ads})$, where nf_{ads} and nf_{des} are the Freundlich slopes for the sorption and desorption isotherms, respectively (O´Connor *et al.*, 1980).

RESULTS AND DISCUSSION

Characteristics of the sorbents

The higher basal spacings of the alkylammonium exchanged montmorillonites compared to the pure clays, SW and SA, indicate the formation of interlayered complexes (Table 1). The basal spacing values of the organoclays at 25°C suggest that ODA and HDTMA cations formed bilayers or pseudo-trimolecular layers in SW montmorillonite ($d_{001} \approx 1.7$-1.8 nm), whereas paraffin-like complexes ($d_{001} > 2.2$ nm) were formed in the case of SA montmorillonite (Jaynes & Boyd, 1991). In the case of the organohydrotalcites, HTDDS and HTDBS, the arrangement of the alkylsulfate anions can be described in terms of paraffin-like complexes, with basal diffractions > 2.2 nm (Table 1).

Sorption-desorption experiments

Triadimefon and imazamox sorption isotherms on all sorbents studied fit the Freundlich equation with $r^2 > 0.97$ (Table 2). Due to the anionic character of imazamox at the pH of the suspensions (pH > 5), this herbicide was not sorbed by the negatively charged surfaces of SW and SA montmorillonites, but it was sorbed by the positively charged hydrotalcite surfaces (Table 2). Triadimefon, that remained uncharged at the pH of the experiments, did not sorb on the high-charge montmorillonite, SA, or hydrotalcite, HT. Some measurable sorption of triadimefon on the low-charge montmorillonite, SW, can be attributed to hydrophobic, non-charged regions present on the surface of this clay, allowing triadimefon molecules to effectively compete with water molecules for those hydrophobic regions (Celis *et al.*, 1999).

Incorporation of large organic ions in the interlayers of montmorillonite and hydrotalcites greatly increased the sorptive capacity of these minerals for imazamox and triadimefon (Table 2). The organic phase in the organoclays and organohydrotalcites changed the nature of the surfaces from hydrophilic to hydrophobic, increasing their affinity for both pesticides. An exception was the imazamox-organohydrotalcite systems, where the repulsions between the interlayer alkylsulfate anions and imazamox anion appeared to dominate and little or no imazamox sorption took place.

Table 2. Imazamox and triadimefon sorption-desorption coefficients on the sorbents studied.

	Imazamox			Triadimefon		
Sorbent	K_f	n_f	H (x 100)	K_f	n_f	H (x100)
SW	0[†]	-	-	88 ± 7[‡]	1.65 ± 0.07	50
SA	0	-	-	0	-	-
HT	34 ± 3	0.97 ± 0.07	-	0	-	-
SW-ODA$_{50}$	100 ± 1	0.93 ± 0.01	3	630 ± 30	0.83 ± 0.03	66
SW-ODA$_{100}$	117 ± 6	0.88 ± 0.04	9	530 ± 20	0.85 ± 0.03	46
SW-HDTMA$_{100}$	36 ± 5	0.88 ± 0.10	28	1420 ± 30	0.88 ± 0.01	89
SA-ODA$_{100}$	115 ± 2	0.93 ± 0.01	3	1400 ± 50	0.78 ± 0.02	19
SA-HDTMA$_{100}$	92 ± 2	0.99 ± 0.01	32	17340 ± 750	0.97 ± 0.02	-[§]
HTDDS	0	-	-	4150 ± 370	1.02 ± 0.05	74
HTDBS	13 ± 2	0.93 ± 0.11	-	1630 ± 20	0.99 ± 0.01	119

[†] no measurable sorption; [‡] value ± standard error; [§] ill-defined desorption isotherms due to very high sorption on these sorbents.

Depending on the sorbent, triadimefon sorption on organoclays and organohydrotalcites was 5 to 200 times greater than that observed for imazamox. For imazamox, the highest sorptive capacity was displayed by the primary ODA-organoclays. The linear conformation of ODA probably allowed easier hydrophobic interaction in the outer organic interlayer. Additional polar interaction of the monosubstituted ammonium group with the carboxylate group of imazamox may have also occurred (Hermosín & Cornejo, 1993; Zhao *et al.*, 1996). For triadimefon, the highest sorptive capacity was displayed by the quaternary SA-HDTMA organoclay. The very high triadimefon sorption on this organoclay suggests interlayer sorption, probably facilitated by the large basal spacing of this clay (2.4 nm) and the presence of room available for sorption between the large tetrasubstituted HDTMA cations.

While sorption irreversibility for triadimefon was intermediate to low depending on the sorbent (intermediate to high H values in Table 2), a high irreversibility of the sorption-desorption process was observed for imazamox in most cases (low H values in Table 2). Reversible behaviour would be desirable in the use of sorbents for slow release formulations, whereas irreversible sorption would be advantageous for pollutant immobilisation of already contaminated soils. For both pesticides, the lowest H values, indicating high irreversibility, correspond to ODA-organoclays. In the case of imazamox, H values for ODA-organoclays were particularly low. Polar and even coulombic interactions between imazamox anions and the ammonium groups of ODA cations (Hermosín & Cornejo, 1993; Zhao *et al.*, 1996) have stabilised the binding of imazamox to the organoclays, thus decreasing desorbability.

pH effect on sorption

Triadimefon and imazamox sorption on organoclays increased with decreasing pH (Table 3). The increase in sorption with decreasing pH was particularly pronounced in the case of imazamox due to formation of molecular imazamox and partition of the neutral imazamox species within the interlayer organic phase of the organoclays. pH also affected imazamox desorption.

Table 3. Effect of pH on triadimefon and imazamox sorption by organoclays.

Organoclay	Initial pH	Final pH		% sorption	
		Imazamox	Triadimefon	Imazamox	Triadimefon
SW-ODA$_{100}$	4.5	6.1	5.4	21	30
	3.0	5.4	3.4	49	54
	2.5	2.9	2.5	73	54
SA-ODA$_{100}$	4.5	6.1	5.6	20	60
	3.0	5.6	4.1	67	89
	2.5	3.3	2.6	87	88

Fig. 1 shows the percentage of imazamox that remained sorbed after successive desorption cycles from the highest equilibrium point of the sorption isotherm. After the second desorption cycle, higher amounts of imazamox was desorbed at pH 3 compared to pH 7. This indicates that neutral imazamox adsorbs through a weaker interaction mechanism, so that desorption is easier. Most likely, contribution of ionic attraction to the binding mechanism was no longer operative in the sorption of the neutral species.

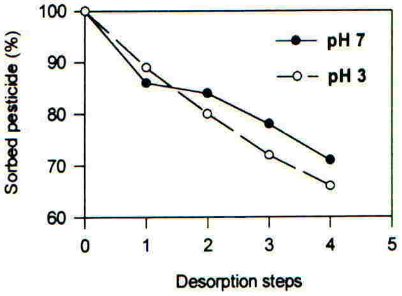


Figure 1. Imazamox desorption from SW-ODA$_{50}$ at pH 3 and pH 7.

CONCLUSIONS

The results of this work show that, depending on pH and selecting the characteristics of the sorbent, organoclays and organohydrotalcites may find application as filters for water decontamination and slow release formulations of pesticides similar to imazamox and triadimefon.

ACKNOWLEDGEMENTS

This work has been partially supported by the CICYT project AMB96-0445-CO2-01 and by the Research Group RNM124 of Junta de Andalucía.

REFERENCES

Celis R; Koskinen W C; Hermosín M C; Cornejo J (1999). Sorption and desorption of triadimefon by soils and model soil colloids. *Journal Agricultural and Food Chemistry* **47**, 776-781.

Darvari R; Hasirci V (1996). Pesticide and model drug release from carboxymethyl cellulose microspheres. *Journal Microencapsulation* **13**, 9-24.

Hermosin M C; Cornejo J (1992).Removing 2,4-D from waters by organoclays *Chemosphere* **24**: 1493-1504.

Hermosín M C; Cornejo J (1993). Binding mechanisms of 2,4-dichlorophenoxyacetic acid by organoclays. *Journal Environmental Quality* **22**, 325-331.

Jaynes W F; Boyd S A (1991). Clay mineral type and organic compound sorption by hexadecyltrimethylammonium-exchanged clays. *Soil Science Society American Journal* **55**, 43-48.

O´Connor G A; Wierenga P J; Cheng H H; Doxtader K G (1980). Movement of 2,4,5-T through large soil columns. *Soil Science* **130**, 157-162.

Reichle W T (1986). Synthesis of anionic clay minerals (mixed metal hydroxides, hydrotalcite). *Solid State Ionics* **22**, 135-141.

Zhao H; Jaynes W F; Vance G F (1996). Sorption of the ionizable organic compound dicamba (3,6-dichloro-2-methoxybenzoic acid) by organo-clays. *Chemosphere* **33**, 2089-2100.

Influence of environmental conditions and soybean agrotechniques on detoxification of herbicides in soil

A A Peneva
Plant Protection Institute, 2230 Kostinbrod, Bulgaria

ABSTRACT

The detoxification of imazaquin, imazethapyr, alachlor, metolachlor, acetochlor, dimethanamid, trifluralin and pendimethalin was studied after application to soybean crops grown on chernozem and alluvial soils. The influence of soil temperature and moisture as well as agrotechniques were investigated over the period of 1993-1998. In this paper, data is presented that correlates herbicide degradation with soil temperature and moisture.

INTRODUCTION

The detoxification of herbicides in soil and the quantity of residues depend on a range of factors including, soil type and structure, pH, organic matter (o. m) content (Stougaard et al., 1990), quantity and frequency of rainfall (Milanova & Grigorov, 1996), soil temperature and moisture (Peneva, 1996, 1997), microbial activity, and tillage techniques (Mills & Witt, 1991). Herbicide behavior in soil can be assessed using different bioassays (Reisler, 1972; Streibig, 1988). In this study, the influence of soil temperature and rainfall on the rates of degradation of herbicides over several years were investigated using bioassays to monitor residual herbicide in soil. The effects of fertilizer application and tillage techniques using soybean as a model system were also investigated.

METHODS AND MATERIALS

During 1993-1994, two field trials were conducted in soybean crops grown on a chernozem soil containing 3.31% o.m., 44% clay, 50% sand with a pH of 6. In the first trial, half of the area was fertilized with 50 kg/ha nitrogen and the other half was left unfertilized. In all trials, herbicides were applied with a hand sprayer (Matabi, model Merk 5, nozzle type 1A, operating pressure 2 bar, 0.4 liters/min). The water volume applied was 1000 liters/ha and the plot size was 5 x 2 m^2.

In the second trial (without fertilization), soybean was sown in half the area with a row spacing of 70-cm and with 24-cm row spacing in the other half. The following herbicides were applied (all doses in a.i./ha); 0.96 trifluralin (ppi), 2.0 metolachlor, 1.92 alachlor (pre-em), 0.38 (ppi) imazaquin, and 0.38 (pre-em.) imazaquin. Soil samples were removed with a spade from a vertical profile of layers 0-5 and 5-10-cm depth 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 days after treatment (DAT).

During 1995-1998, field trials were conducted on an alluvial soil containing 1.07% o.m., 20% clay, 72% sand with a pH of 7. The area was left unfertilized and the following herbicides were applied pre-em.; 0.30 imazaquin, 0.10 imazethapyr, 2.30 acetochlor, 1.32 pendimethalin, and 1.35 dimethanamid. Soil samples were taken from 0-5, 5-10, 10-20 and 20-30-cm depths 2 h and 15, 30, 45, 60, 80, 100, 120, and 150 DAT. Mean soil temperatures at each depth and rainfall were measured. Herbicide residues were determined using bioassays (radish and wheat) by comparing root length as well as above-ground fresh and dry biomass of plants grown in field soils with plants grown in untreated soils spiked with each herbicide (0, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ppm).

RESULTS AND DISCUSSION

Detoxification of imazaquin applied ppi was more rapid than when applied pre-em. Degradation rates were faster in fertilized as opposed to unfertilized soils because of increased microbial activity in the fertilized soil. The greatest rate of degradation occurred during the first 60 DAT. More residues were detected at 5-10 cm depth (Table 1).

Table 1. Residual amounts (mg/kg) of imazaquin after application of 0.38 kg ai/ha to a chernozem soil (1993-1994).

DAT	Fertilized				Unfertilized			
	ppi		pre - em		ppi		pre -em	
	Depth (cm)							
	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10
15	0.49	0.60	1.03	1.06	0.79	0.78	1.72	1.44
30	0.17	0.47	0.55	0.85	0.53	0.62	1.30	1.23
45	0.14	0.28	0.42	0.55	0.35	0.43	0.92	1.03
60	0.11	0.16	0.24	0.33	0.12	0.14	0.67	0.74
75	0.05	0.07	0.14	0.30	0.05	0.06	0.18	0.23
90	0.04	0.06	0.07	0.13	0.04	0.05	0.11	0.10
105	0.03	0.04	0.04	0.05	0.03	0.04	0.09	0.07
120	0.02	0.03	0.03	0.03	0.02	0.03	0.05	0.05
135	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.02
150	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01

Detoxification of trifluralin, metolachlor and alachlor was more rapid in soil where row spacing was wide as opposed to narrow (70 vs. 24-cm). Regardless of row spacing, degradation of trifluralin was similar to that of metolachlor in that more residues were detected in the upper layers of the soil. However, in the case of alachlor, more residues were detected at a depth of 0-5 cm in the soil with narrow row spacing between soybean (Table 2).

Table 2. Residual amounts (mg/kg) of trifluralin, metolachlor and alachlor in chernozem soil.

	Wide-row spacing (70-cm)						Narrow-row spacing (24-cm)					
DAT	Trifluralin		Metolachlor		Alachlor		Trifluralin		Metolachlor		Alachlor	
	Depth (cm)											
	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10
15	0.97	0.93	1.66	1.35	1.36	1.45	1.48	1.16	1.90	1.59	1.75	1.49
30	0.63	0.58	1.55	1.23	1.19	1.27	1.06	0.70	1.73	1.44	1.54	1.26
45	0.57	0.55	1.25	1.08	1.01	1.14	0.84	0.65	1.43	1.26	1.15	1.00
60	0.32	0.35	1.00	0.56	0.80	0.90	0.73	0.60	1.30	1.18	0.83	0.75
75	0.20	0.13	0.53	0.43	0.35	0.63	0.63	0.56	1.11	1.11	0.57	0.55
90	0.17	0.11	0.32	0.30	0.09	0.18	0.54	0.45	1.02	0.97	0.29	0.18
105	0.15	0.10	0.21	0.19	0.06	0.17	0.49	0.40	0.58	0.66	0.21	0.16
120	0.13	0.07	0.11	0.09	0.04	0.07	0.27	0.22	0.37	0.32	0.14	0.07
135	0.09	0.05	0.06	0.04	0.02	0.06	0.16	0.12	0.19	0.19	0.07	0.05
150	0.04	0.02	0.03	0.03	0.01	0.02	0.07	0.06	0.12	0.11	0.04	0.03

There was a direct relationship between soil temperatures and the dynamics of detoxification. Furthermore, soil moisture was a major factor that influenced the movement of the herbicides (Figures 1,2). At high soil moisture, and low temperatures (rainfall over 300 liters/m^2 and temperatures under 25°C), in 1995 (Figure 1), the intensive detoxification was over by 60-80 DAT. At high soil moisture and temperatures (200-300 liters/m^2 and 25-30°C), in 1996 and 1997 (Figures 1 & 2), the intensive degradation was over by 60 DAT. At low soil moisture and high temperatures (in 1994) the intensive detoxification ended by 45-60 DAT.

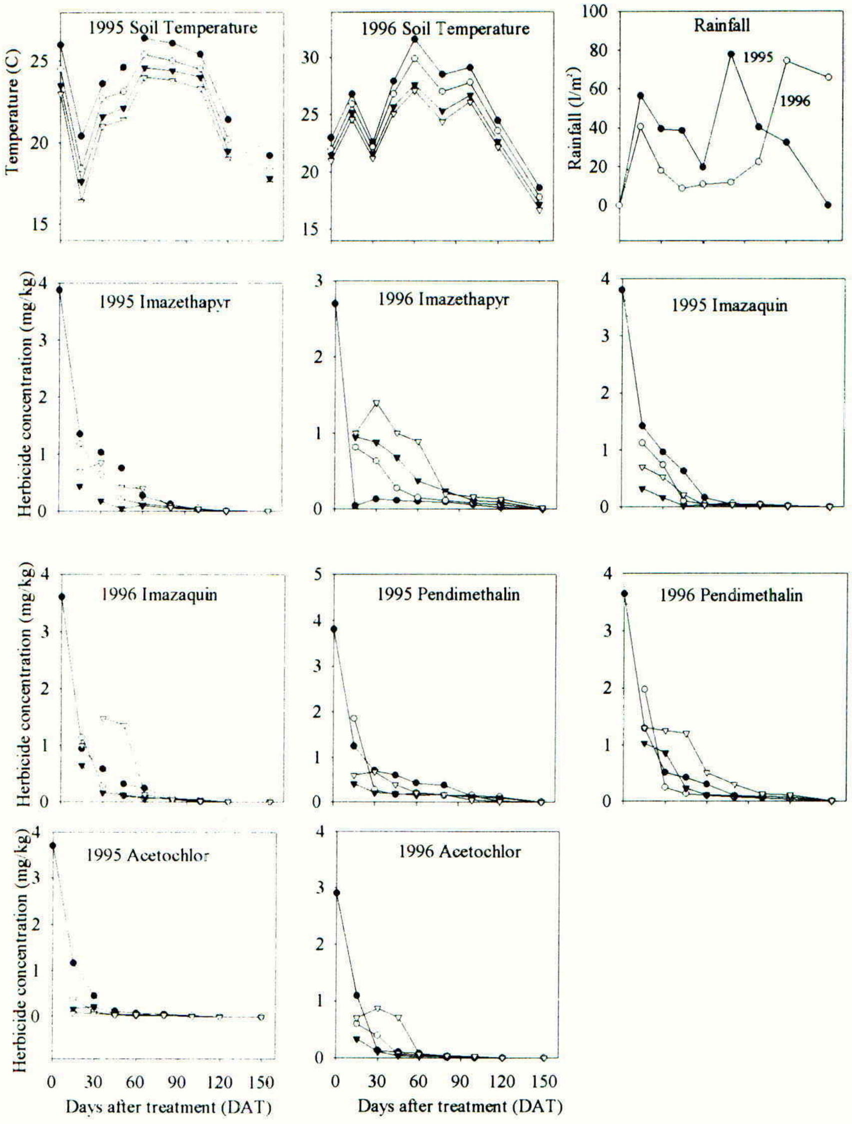


Figure 1. Degradation of herbicides in 1995 and 1996. Mean soil temperature and rainfall in 1995 and 1996 are also included. Various soil depths are indicated by symbols: (●) 0-5 cm, (o) 5-10 cm, (▼) 10-20 cm and (▽) 20-30 cm.

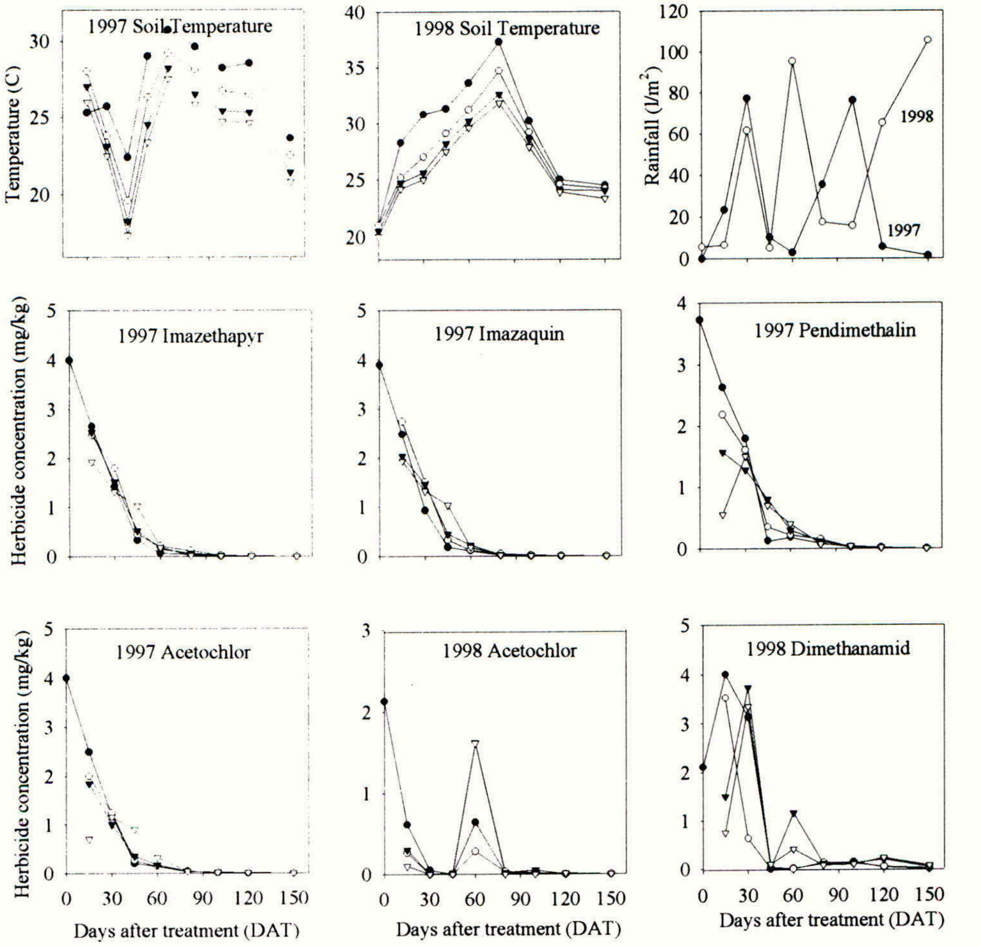


Figure 2. Degradation of herbicides in 1997 and 1998. Rainfall and mean soil temperatures during those years are also illustrated. Various soil depths are indicated by symbols: (●) 0-5 cm, (o) 5-10 cm, (▼) 10-20 cm and (∇) 20-30 cm.

In conclusion, addition of fertilizer increased degradation. Soil moisture content and temperature also influenced the persistence of all the herbicides studied. Degradation was greatest at high temperatures/low soil moisture content (40-60 days), followed by high temperature/high soil moisture content (60 days), and finally by low temperature/high soil moisture content (60-80 days).

It is hypothesized that at high temperature and moderate to high soil moisture content, water evaporates from the soil surface causing upward translocation of the herbicides to the upper layers of soil. Once near the soil surface, high temperature and fertility increase microbial degradation. Conversely, at low temperatures and moderate to high soil moisture content, there is little evaporation of water from the soil hence no upward herbicide movement. Consequently, the herbicides remain deeper in the soil profile where there is less microbial degradation. In most cases, 150 DAT the residual amounts of all herbicides were insignificant at all depths up to 30 cm.

At high soil moisture and low temperatures, more residues of dimethanamid, imazethapyr and pendimethalin than the other herbicides remain in the soil. These residues may pose a threat to subsequent rotational crops.

REFERENCES

Milanova S; Grigorov P (1996). Movement and persistence of imazaquin, oxyfluorfen, fluorochloridone and terbacil in soil. *Weed Research* **36**, 31-36.

Mills J A; Witt W W (1991). Dissipation of imazaquin and imazethapyr under conventional and no-tillage soybean (*Glycine max*). *Weed Technology* **5**, 586-591.

Peneva A (1996). Influence of temperature and humidity on detoxication and translocation dynamics of herbicides in soil. In: *Modern plant protection.* Proceedings of a scientific session "60 years Plant Protection Institute", pp. 525-531.

Peneva A (1997). Afteraction of imazaquin and imazethapyr, applied in soybean, on wheat, maize and sunflower. In: *Scientific Practical Conference on "Ecological problems of agriculture" Agroeco '97.* Higher School of Agriculture-Plovdiv, Scientific Works, vol. XLII, book 3-Part I, 1997, pp. 315-322.

Reisler A (1972). Bioassay of soil samples for content of photosynthesis inhibiting herbicides. Instituut voor Biologischen Scheikundig Onderzoek Van Landbouwgewassen, Wageneingen 61.

Stougaard R H; Shea P J; Martin A R (1990). Effect of soil type and pH on adsorption, mobility and efficacy of imazaquin and imazethapyr. *Weed Science* **38**, 67-73.

Streibig J C (1988). Herbicide bioassay. *Weed Research* **28**, 479-484.

A rapid, sensitive bioassay method for sulfonylurea herbicides

E Hernández-Sevillano, M Villarroya, M C Chueca, J L Alonso-Prados, J M García-Baudín
Área Protección Vegetal INIA, Carretera La Coruña Km 7.5, Madrid, Spain

ABSTRACT

The detection of soil herbicide residues whose concentration is lower than 0.05 mg a.i. kg^{-1} of soil, cannot usually be realised by means of conventional analytical methods. Therefore, it is necessary to use sensitive species in bioassays as suitable screening tests to determine the presence of phytotoxic residues in soil. Bioassays with sunflower (*Helianthus annuus* L.) cv. Albani have been carried out to detect chlorsulfuron, sulfosulfuron and triasulfuron residues in two different soils: sandy loam soil (pH 7.3) and sandy soil (pH 6.1). Root and shoot length, and shoot fresh weight were the parameters measured in sunflower plants. All these parameters were significantly affected by these three herbicides at $5 \cdot 10^{-4}$ mg a.i. kg^{-1} of sandy soil, 15 days after the treatment. In sandy loam soil, root and shoot length were the most sensitive parameters affected and were inhibited at least at $5 \cdot 10^{-3}$ mg a.i. kg^{-1} of soil by the three herbicides. Triasulfuron was detected by sunflower plants at lower doses. Another assay using sunflower in hydroponic culture was also carried out to detect phytotoxic residues of the same three herbicides, measuring the same parameters 7 days after the treatment. Significant inhibition of root length (55% compared with the control) was observed at $1 \cdot 10^{-5}$ ppm of sulfosulfuron. With triasulfuron and chlorsulfuron the same level of root length inhibition (around 50%) was detected at $5 \cdot 10^{-5}$ ppm and $1 \cdot 10^{-4}$ ppm, respectively.

INTRODUCTION

Triasulfuron, chlorsulfuron and sulfosulfuron are members of the sulfonylurea group herbicides. Triasulfuron and chlorsulfuron are used in several countries for weed control in cereals. Sulfosulfuron is a new sulfonylurea herbicide for control of some grass and some annual broadleaf weeds in wheat and could be used to control volunteer barley in winter wheat crops (Shinn *et al.*, 1999). These herbicides are effective against a wide range of weeds at rates between 4 and 20 g a.i. ha^{-1}.

The use of the sulfonylurea herbicides at low dose (<50 g a.i. ha^{-1}) produces not only the typical environmental problems caused by the accumulation of chemical products in crop lands, but also the problem of low quantities of non-degradable or slow-degradable residues in soils that can affect sensitive rotational crops. The three herbicides aforementioned are highly active in soil and some sensitive rotational crops have been damaged by the remaining residue in soil (Walker & Welch, 1989; Kotoula-Syka *et al.*, 1993).

The degradation, fate and behaviour of sulfonylurea herbicides in soil have been widely studied, and their half-life in soil can vary significantly depending on pH, temperature, moisture content, texture and organic matter content of the soil (Sarmah *et al.*, 1999; Blacklow

& Pheloung, 1991). However, there exists a lack of data that links the residue of sulfonylureas in soil and the potential risk for sensitive rotational crops. In order to assess this risk for sensitive rotational crops it is necessary to know the response of these crops to several concentrations of these herbicides. On the other hand the amount of sulfonylurea residue in soil is so low that they are very difficult to determine using conventional analytical methods. Consequently, the development of bioassays using sensitive species are necessary to detect the presence of phytotoxic levels of residue in soil (Stork & Hannah, 1996; Rahman *et al.*, 1993). Recently, the regulatory organizations of the use of pesticides have proposed this type of bioassays to determine the presence of low levels of residues in soil.

Several bioassay methods for sulfonylurea herbicides have been reported using several plant species as lentil (Blacklow & Pheloung, 1991), lettuce (Walker & Welch, 1989), sunflower (Günther *et al.*, 1993), maize (Vicari *et al.*, 1994), pea and lupin (Stork & Hannah, 1996). In this study we show the responses of sunflower to different concentrations of sulfonylurea herbicides in hydroponic culture and to sulfonylurea soil residues in two kinds of soil, in order to use this system as a bioassay to detect phytotoxic levels of sulfonylureas in field soils in which cereal crops were grown.

MATERIALS AND METHODS

Both types of bioassays (hydroponic and soil culture) were carried out using sunflower (*Helianthus annuus* L.) cv. Albani. Sunflower was the most sensitive plant species in a previous bioassay carried out using soil from wheat crops treated with sulfonylureas (data not shown). The herbicides used in both cases were the sulfonylureas chlorsulfuron, sulfosulfuron and triasulfuron. All the assays took place in a growth chamber with 16 h of light (illumination 110 $\mu Em^{-2}s^{-1}$) at 22±1°C and 8 h of darkness at 16±1°C. All the sunflower seeds were previously germinated in Petri dishes on filter paper discs soaked in deionized water for some days.

Hydroponic assay

After three days in the Petri dish, four seedlings were placed on a plastic grid, which was placed into a 220 ml plastic container wrapped in black cardboard. Each container was filled with respective herbicide dose, dissolved in 175 ml of Hewitt nutrient solution (pH 5.2-5.3). The doses of the three herbicides were $0 - 1{\cdot}10^{-5} - 5{\cdot}10^{-5} - 1{\cdot}10^{-4}$ ppm a.i. There were five replicates per dose. After 2 and 4 days, the containers were refilled with Hewitt nutrient solution so as to keep solution at the grid level and cover the plant roots. On the seventh day after the treatment the following parameters were measured: root and shoot length, and shoot fresh weight.

Soil assay

Soils from two different sites were used in this part of the assay. Both soils were air dried and passed through a 5 mm sieve. The physical and chemical properties of the sandy soil were: 86.8% sand, 7.9% silt and 5.3% clay; 1.0% organic matter and pH 6.1 (1:2.5 soil to water). The properties of the sandy loam soil were: 59.8% sand, 26.9% silt and 13.3% clay; 3.2% organic matter and pH 7.3 (1:2.5 soil to water).

After four days in the Petri dish, one seedling was planted in a 5.5-cm diameter by 5-cm high plastic pot filled with soil. Each herbicide dose was dissolved in deionized water and was mixed homogeneously with the quantity of soil needed for each treatment, in a volume of water required to wet the soil completely but not to lose herbicide by leaching. The doses employed for the three herbicides were $0 - 5 \cdot 10^{-4} - 1 \cdot 10^{-3} - 5 \cdot 10^{-3}$ mg a.i. kg^{-1} of soil. Since the density of each soil was different, the pots containing sandy soil were filled with 116 g of soil and moistened with 17 ml of the correspondent herbicide solution, while the ones containing sandy loam soil had 94 g moistened with 18 ml. There were ten replicates per dose. After 2 days, the pots were sub-irrigated with deionized water so as to keep the soils wet. The plants were sub-irrigated as much as needed during the experience. On the fifteenth day after the treatment, the assay was finished and the roots of the plants were washed with water in order to clean them. The following parameters were measured on the plants: root and shoot length, and shoot fresh weight.

Means of each treatment were analysed using ANOVA and were compared by Newman-Keuls multiple range test at significance levels of 1 and 5% for the hydroponic and soil assays, respectively.

RESULTS AND DISCUSSION

Hydroponic assay

The response of hydroponically grown sunflower plants to different concentrations of the three herbicides was evaluated. Root length was the most sensitive biological parameter (Table 1). Shoot length and shoot fresh weight were also measured, but there was no difference between control and treated plants.

Root length was significantly reduced by 55% compared with the control at $1 \cdot 10^{-5}$ ppm of sulfosulfuron. Sunflower plants were less sensitive to triasulfuron and chlorsulfuron, compared with sulfosulfuron, as significant differences were achieved at $5 \cdot 10^{-5}$ and $1 \cdot 10^{-4}$ ppm with a root growth being inhibited by 55% and 51%, respectively.

Table 1: Effect of sulfonylurea herbicides at different concentrations on sunflower plants in hydroponic assay (% compared with untreated control)[1].

DOSES (ppm)	SULFOSULFURON		TRIASULFURON		CHLORSULFURON	
	% Root length	% Shoot length	% Root length	% Shoot length	% Root length	% Shoot length
0	100 a	100 a	100 a	100 a	100 a	100 a
$1 \cdot 10^{-5}$	45 b	91 a	88 a	103 a	98 a	82 a
$5 \cdot 10^{-5}$	45 b	78 a	45 b	74 a	74 a,b	73 a
$1 \cdot 10^{-4}$	31 b	71 a	33 b	81 a	49 b	67 a

[1] Means of each treatment and each parameter were analysed using ANOVA and compared by Newman-Keuls multiple range test. Letters after value indicate significant differences at $P \leq 0.01$.

Our results show that under hydroponic conditions the effects of the three tested sulfonylurea herbicides on sunflower root length were in the following order: chlorsulfuron < triasulfuron < sulfosulfuron.

Soil assay

The response of sunflower plants to different concentrations of the three herbicides under study was also evaluated in soil. Like in hydroponic culture, root length was also a good biological indicator to detect low concentrations of these herbicides in soil (Table 2). Shoot length also showed a significant growth inhibition in soil, so it could be used as biological indicator. On the contrary, shoot fresh weight was a less sensitive parameter and could not be used to detect significant differences due to the herbicides.

The assays also showed that effects were higher in the sandy soil than in the sandy loam. Both parameters considered were significantly different at $5 \cdot 10^{-4}$ mg a.i. kg^{-1} of sandy soil, while in the sandy loam the differences varied from one herbicide to another and were noticed at a higher dose than in the sandy soil. These differences among the responses of sunflower in both soils were due to the higher organic matter and clay content in the sandy loam soil, which seem to influence herbicide bioavailability, since pH is similar in both soils.

In sandy soil, sunflower plants were equally sensitive to all the herbicides, at a level of $5 \cdot 10^{-4}$ mg a.i. kg^{-1} of soil. Regardless of the herbicide, root length was reduced by 33%-51% and shoot length by 42%-54% compared with the control.

In sandy loam soil, $5 \cdot 10^{-3}$ mg a.i. kg^{-1} of soil can be considered a very reliable limit of detection for the three sulfonylurea herbicides in soil, when root and shoot length are taken as relevant parameters. However, the data show that in the case of triasulfuron, sunflower roots and shoots were affected by a 5 times lower dose ($1 \cdot 10^{-3}$ mg a.i. kg^{-1} of soil). This limit of detection may be even lower when shoot length is taken into account as the ANOVA shows a shoot growth inhibition by 29% at a 10 times lower dose ($5 \cdot 10^{-4}$ mg a.i. kg^{-1} of soil). But this 29% growth inhibition is on the edge of what may be considered biologically significant due to the natural biological variation.

Finally, we can prove that this is an acceptable method to detect lower sulfonylurea concentrations in soil than the most realistic determination limits of sulfonylureas achieved by conventional analytical methods, which is 0.01 ppm.

Table 2: Effect of sulfonylurea herbicides at different concentrations on sunflower plants in soil assay (% compared with untreated control)[1].

SULFOSULFURON

DOSES (mg/kg)[2]	**SANDY LOAM**			**SANDY**		
	% Root length	**% Shoot length**	**% Shoot fresh weight**	**% Root length**	**% Shoot length**	**% Shoot fresh weight**
0	100 a	100 a	100 a	100 a	100 a	100 a
$5 \cdot 10^{-4}$	88 a,b	88 a	102 a	67 b	58 b	82 b
$1 \cdot 10^{-3}$	75 b	62 b	83 a	62 b	35 c	75 b
$5 \cdot 10^{-3}$	26 c	31 c	60 b	21 c	31 c	73 b

TRIASULFURON

DOSES (mg/kg)[2]	**SANDY LOAM**			**SANDY**		
	% Root length	**% Shoot length**	**% Shoot fresh weight**	**% Root length**	**% Shoot length**	**% Shoot fresh weight**
0	100 a	100 a	100 a	100 a	100 a	100 a
$5 \cdot 10^{-4}$	88 a	72 b	83 b	67 b	46 b	74 b
$1 \cdot 10^{-3}$	52 b	54 c	71 c	49 b	38 b	72 b
$5 \cdot 10^{-3}$	20 c	34 d	59 d	27 c	37 b	72 b

CHLORSULFURON

DOSES (mg/kg)[2]	**SANDY LOAM**			**SANDY**		
	% Root length	**% Shoot length**	**% Shoot fresh weight**	**% Root length**	**% Shoot length**	**% Shoot fresh weight**
0	100 a	100 a	100 a	100 a	100 a	100 a
$5 \cdot 10^{-4}$	89 a	83 a	94 a	49 b	54 b	72 b
$1 \cdot 10^{-3}$	85 a	79 a	102 a	41 b,c	45 b	75 b
$5 \cdot 10^{-3}$	37 b	68 a	80 a	28 c	46 b	58 c

[1] Means of each treatment and each parameter were analysed using ANOVA and compared by Newman-Keuls multiple range test. Letters after value indicate significant differences at $P \leq 0.05$.

[2] The doses are expressed in mg a.i. kg^{-1} of soil.

ACKNOWLEDGEMENTS

We wish to thank DuPont, Monsanto and Novartis for the gift of chlorsulfuron, sulfosulfuron and triasulfuron, respectively.

REFERENCES

Blacklow W M; Pheloung P C (1991). Sulfonylurea herbicides applied to acidic sandy soils: a bioassay for residues and factors affecting recoveries. *Australian Journal of Agricultural Research* **42**, 1205-16.

Günther P; Pestemer W; Rahman A; Nordmeyer H (1993). A bioassay technique to study the leaching behaviour of sulfonylurea herbicides in different soils. *Weed Research* **33**, 177-185.

Kotoula-Syka E; Eleftherohorinos I G; Gagianas A; Sficas A G (1993). Phytotoxicity and persistence of chlorsulfuron, metsulfuron-methyl, triasulfuron and tribenuron-methyl in three soils. *Weed Research* **33**, 355-367.

Rahman A; James T K; Günther P (1993). Bioassays of soil applied herbicides. In: *Proceedings International Symposium Indian Society of Weed Science* **1**, 95-106.

Sarmah A K; Kookana R S; Alston A M (1999). Degradation of chlorsulfuron and triasulfuron in alkaline soils under laboratory conditions. *Weed Research* **39**, 83-94.

Shinn S L; Thill D C; Price W J (1999). Volunteer Barley (*Hordeum vulgare*) control in winter wheat (*Triticum aestivum*) with MON 37500. *Weed Technology* **13**, 88-93.

Stork P; Hannah M C (1996). A bioassay method for formulation testing and residue studies of sulfonylurea and sulfonanylide herbicides. *Weed Research* **36**, 271-281.

Vicari A; Catizone P; Zimdahl R L (1994). Persistence and mobility of chlorsulfuron and metsulfuron under different soil and climatic conditions. *Weed Research* **34**, 147-155.

Walker A; Welch S J (1989). The relative movement and persistence in soil of chlorsulfuron, metsulfuron-methyl and triasulfuron. *Weed Research* **29**, 375-383.

Toxicity tests for assessment of pesticide effects on aquatic plants

J Davies, H F Pitchford, J R Newman and M P Greaves
IACR - Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS41 9AF, UK

ABSTRACT

In response to concerns that current risk assessment procedures may not adequately reflect the long-term risk of pesticides to non-target aquatic plants, the results of standard toxicity tests, based on *Lemna minor,* have been compared with responses seen in alternative tests, based on *Myriophyllum spicatum,* a submerged dicotyledon, *Lagarosiphon major*, a submerged monocotyledon, and *Glyceria maxima,* an emergent monocotyledon. For this purpose, short-term, glasshouse-based studies were conducted to determine the relative sensitivity of each species to selected herbicides, while a long-term, outdoor, mesocosm study is in progress to evaluate the ecological relevance of glasshouse-generated results. Large differences were observed between the sensitivities of these species which indicated that *Lemna* is not the most sensitive test species for all herbicides. Furthermore, results from the mesocosm experiment revealed long-term effects that cannot be predicted from short-term tests and may have implications for species viability in subsequent seasons. The implications for the selection of appropriate species for use in toxicity tests will be discussed.

INTRODUCTION

Under current risk assessment procedures, the potential effect of a herbicide on a non-target species is quantified by assessing the toxicity of the compound and the level of exposure likely to be encountered in the environment. Toxicity is estimated from short-term, single species, laboratory-based tests which take the form of dose response experiments and enable calculation of a response parameter, such as the EC_{50} (concentration causing 50% effect). In the case of aquatic plants, the duckweeds (*Lemna* sp.) are used as indicator species in 7 or 14 day tests incorporating a single exposure to the herbicide. Meanwhile, exposure is expressed as a predicted environmental concentration (PEC) and, for purposes of risk assessment in EU countries, initial surface water PECs for hydraulic sprayers, are calculated assuming 4% spray drift into a 30 cm deep, static water body. These parameters are then used to calculate a toxicity exposure ratio (TER) which is expressed as the ratio of EC_{50} to the PEC and should be >10 for risk to be judged acceptable. Where the TER falls below this safety margin, additional testing using more realistic exposure scenarios may be requested or risk mitigation steps may be implemented by regulatory authorities (Campbell & Hoy, 1996).

This risk assessment process assumes that *Lemna* is a valid indicator species for all aquatic plants and that a safety factor of 10 will allow for inter-species differences in sensitivity. Furthermore, it also assumes that short-term effects observed under laboratory conditions can be used to predict long-term consequences in the environment, (Chapman *et al*.,1998). In order to test the validity of these assumptions and establish whether this approach provides adequate protection for non-target aquatic plants, the performance of a standard test based on *Lemna minor* (common duckweed; floating monocotyledon) is currently being compared with responses in alternative tests, based on *Myriophyllum spicatum* (spiked watermilfoil; submerged dicotyledon), *Lagarosiphon major* (curly waterweed; submerged monocotyledon) and *Glyceria maxima* (reed sweet-grass; emergent monocotyledon). For this purpose, short-term, glasshouse-based studies are being conducted in order to assess the relative sensitivity of each species to selected herbicides, while a long-term, outdoor, mesocosm study is in progress to evaluate the ecological relevance of glasshouse-generated results.

METHODS AND MATERIALS

Mesocosm construction and plant material

Mesocosms (2.35m width, 4.86m length, 65 cm depth) were constructed in April 1998 and lined with black membrane. Mesocosms were filled to 30 cm with bore-hole water and maintained at this depth until treatment. After this time, depths were allowed to fluctuate naturally up to a maximum of 60 cm, at which point mesocosms were drained to their original depth. All species were planted in plastic pots containing sterilized, sandy-loam soil with a lower layer of peat (1-2 cm) and a soil surface covering of pea shingle. In the case of *G. maxima*, 18 month-old seedlings were transplanted in 10 l pots and positioned such that the soil surface was exposed above the water surface. In the case of *L. major* and *M. spicatum*, bunches of six stems, 10-15 cm in length, were planted in 3 l pots such that >5 cm stem was below the soil surface while the remainder was entirely submerged below the water surface. Pots (six *L. major*, six *G. maxima* and five *M. spicatum*) were placed in each mesocosm and allowed to establish for 8 weeks prior to herbicide treatment.

Herbicide treatments

Mesocosms were treated with one of six herbicides, selected to represent a range of chemistries, selectivities and diferent modes of action (Table 1). Each was applied at doses equivalent to the PEC and 10xPEC, *i.e.* 4% and 40% of maximum recommended field rates, to three replicate tanks arranged in a randomized block design. Treatments were applied by knapsack sprayer calibrated to deliver 250 l/ha and were repeated in accordance with the recommended use pattern for each product.

Table 1. Herbicide treatments.

Compound	Product	Maximum recommended field rate (kg/ha)	PEC (μg/ml)
Metsulfuron-methyl	'Ally'	0.006	0.00008
MCPA	'HY-MCPA'	1.750	0.023
Glyphosate	'Roundup ProBiactive'	2.160	0.029
Isoproturon	'Isoproturon 500'	2.500	0.033
Diclofop-methyl	'Hoegrass'	1.701	0.023
Imazamethabenz-methyl	'Dagger'*	0.900	0.012

* applied with Agral @ 500 ml/ha

Assessments and statistical analyses

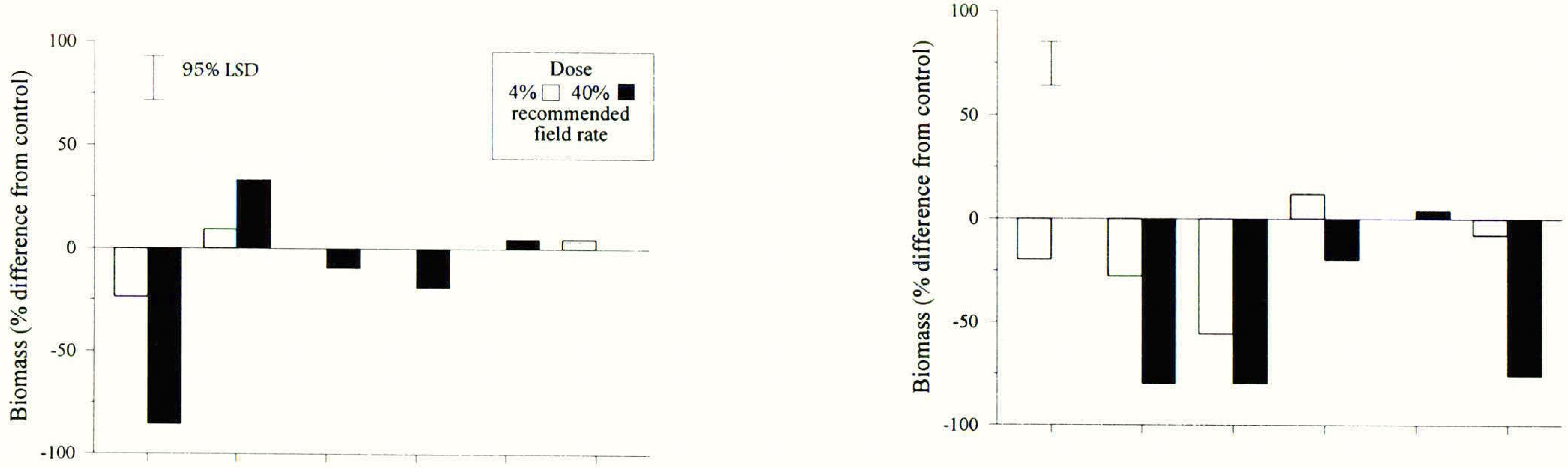
Biomass was estimated visually on a scale of 0-10 where 0 represents 100% mortality and 10 represents healthy growth. Assessments were made weekly from 3 weeks after treatment for 2 months. In the case of *G. maxima*, biomass was estimated relative to control treatments, while the biomass of submerged species was estimated in terms of percentage water surface covered. Flowering was also recorded for all species. For *Lagarosiphon,* additional assessments were made of side-shoot production. For this purpose, 5 x 50 cm lengths of stem were randomly sampled from each mesocosm, 5 weeks after treatment. Each length was blotted dry before being divided into main stem and side-shoot components. Length and weight were then recorded for main stem, while number, lengths and combined weight were measured for side-shoots. Dry weights were recorded after oven drying at 80°C for 3 days. All data was analysed by ANOVA using Genstat 4.1 for Windows. Visual score data is presented in terms of percent difference from control, while shoot number data has been transformed onto a log scale and also expressed relative to control.

RESULTS

Biomass data revealed a wide variation in the sensitivities of each species to the range of herbicides tested and suggested that no single species can be used as a universal indicator for all potential herbicide effects (Figure 1). For example, while *Myriophyllum* responds significantly to isoproturon, MCPA and metsulfuron-methyl, *Glyceria* responds significantly to glyphosate and isoproturon, and *Lagarosiphon* responds only to metsulfuron-methyl.

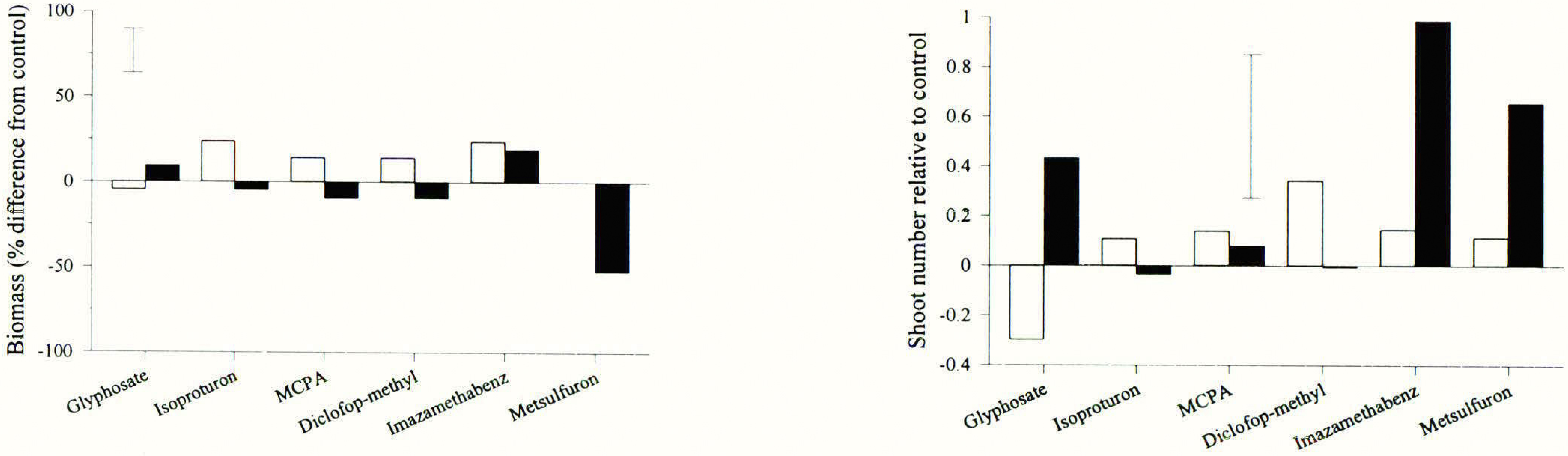
In the case of *Glyceria*, treatment with glyphosate significantly reduced biomass by 24 and 86% at 4 and 40% doses, respectively, while, isoproturon applied at 40% field rate increased biomass by 33%. In contrast, 4 and 40% doses of isoproturon applied to *Myriophyllum*, reduced biomass by 28 and 80%, respectively, while the 40% dose also inhibited flowering in this species. Similar effects were caused by metsulfuron-methyl which reduced biomass by 76% and inhibited flowering when applied at 40% field rate. *Myriophyllum* was also susceptible to inhibition by MCPA which caused severe epinasty within 3 weeks of treatment and reduced final biomass by 56 and 80% at 4 and 40% doses, respectively.

In contrast to *Myriophyllum*, assessments for *Lagarosiphon* suggest that this species is less sensitive, only responding significantly to metsulfuron-methyl with a 52% reduction in biomass at the 40% dose. However, several treatments caused more subtle effects which were not apparent from direct estimates of biomass. In particular, three herbicides produced changes in growth pattern either by causing a proliferation of side-shoots or increasing stem fragmentation. Counts of side-shoot number showed that 40% doses of imazamethabenz-methyl and metsulfuron-methyl, doubled and tripled side-shoot number, respectively (Figure 1d), while MCPA treatments caused growth deformities which led to increased stem fragmentation. Both of these changes in growth pattern increase the potential for the production of vegetative propagules. To assess the viability of these propagules, free-floating *Lagarosiphon* stem fragments were taken from each mesocosm and transplanted into new mesocosms containing fresh water. Visual estimates of biomass over the following 6 months, showed no significant differences between the growth of propagules taken from mesocosms



(a) Glyceria maxima

(b) Myriophyllum spicatum



(c) Lagarosiphon major

(d) Lagarosiphon major

Figure 1. Effect of herbicides on *Glyceria maxima*, *Myriophyllum spicatum* and *Lagarosiphon major* .

which did not suffer changes in growth pattern and those taken from mesocosms treated with imazamethabenz-methyl, metsulfuron-methyl or MCPA. This observation suggests that the increased production of vegetative propagules resulting from herbicide treatment could increase the vegetative reproduction and distribution of *Lagarosiphon*, thus disrupting the species balance within macrophyte communities. In addition to effects on vegetative propagation, two compounds were also found to inhibit flowering in *Lagarosiphon*. As was the case for *Myriophyllum*, plants treated with isoproturon or metsulfuron-methyl at the 40% dose did not produce flowers. Such an effect will prevent sexual reproduction and limit diversification within both species. A summary of these effects is provided in Table 2.

Table 2. Summary of herbicide effects observed in mesocosm study.

Herbicide	*Glyceria*	*Myriophyllum*	*Lagarosiphon*
Glyphosate	$\mathbf{X}_{b}$		
Isoproturon		$\mathbf{X}_{b,f}$	$\mathbf{X}_{f}$
MCPA		$\mathbf{X}_{b}$	$\mathbf{X}_{g}$
Diclofop-methyl			
Imazamethabenz-methyl			$\mathbf{X}_{g}$
Metsulfuron-methyl		$\mathbf{X}_{b,f}$	$\mathbf{X}_{b,f,g}$

X_b reduced biomass; X_f inhibition of flowering; X_g altered growth pattern; - no effect

DISCUSSION

The relative sensitivities of this mesocosm study and a standard toxicity test using *Lemna minor*, are compared in Table 3, where the lowest EC_{50} and NOEC (no-observable effect concentration) parameters estimated from *Lemna* tests, conducted in our laboratory according to procedures recommended by Sims *et al.* (1999), are listed alongside the LOEC (lowest observable effect concentration) for the most sensitive species in the mesocosm study.

Table 3. Comparison of *Lemna* and mesocosm responses to herbicide treatments.

Herbicide	*Lemna* EC_{50} (ppm)	*Lemna* NOEC (ppm)	Mesocosm LOEC (ppm)
Glyphosate	28.0	8.0	0.290
Isoproturon	0.05	0.01	0.033
MCPA	1.2	0.40	0.023
Diclofop-methyl	n.d.	n.d.	>0.230
Imazamethabenz-methyl	0.87	0.33	0.120
Metsulfuron-methyl	n.d.	n.d.	0.0008

n.d. not determined

While this data set is incomplete for diclofop-methyl and metsulfuron-methyl, in the case of glyphosate, MCPA and imazamethabenz-methyl, *Lemna* NOEC parameters are higher than mesocosm LOECs, indicating that *Lemna* tested under laboratory conditions, is less sensitive than at least one of the alternative species tested under mesocosm conditions. In particular, *Glyceria* was at least 27-fold more sensitive to glyphosate, while *Myriophyllum* was 17-fold more sensitive to MCPA and *Lagarosiphon* was approximately three-fold more sensitive to imazamethabenz-methyl. Furthermore, the observed differences in sensitivity for glyphosate and MCPA exceed the safety margin of 10, currently used in TER assessments, which is assumed to allow for differences in sensitivity when extrapolating from indicator species to other non-target species. In the case of isoproturon, although the mesocosm LOEC is greater than the *Lemna* NOEC, it should be noted that the quoted LOEC was the lowest dose applied in the mesocosm study and that the actual effect recorded at this dose was a 28% reduction in biomass. Therefore, while the absence of complete dose response data for isoproturon in the mesocosm experiment prevents a conclusive comparison, it is possible that *Lemna* may also be less sensitive to isoproturon than *Myriophyllum* grown under mesocosm conditions.

CONCLUSIONS

To date, *L. minor*, tested under laboratory conditions, has proved less sensitive than other non-target, aquatic plant species tested under more realistic exposure conditions, to four out of six herbicides representing a range of chemistries, selectivities and modes of action. Furthermore, in two cases, the observed difference in sensitivity exceeds the safety margin of 10 used in TER calculations. This finding suggests that current risk assessment procedures which use *L. minor* as an indicator species, may not provide adequate protection for aquatic plants against all adverse effects of herbicides. In light of these observations, an ideal testing strategy would incorporate all four species or at least provide the option of selecting the species which is most appropriate for the chemistry and exposure scenario of the pesticide under test. Consequently, the development of new toxicity tests based on *Glyceria*, *Lagarosiphon* and *Myriophyllum* is continuing, while the ecological consequences of pesticide effects on vegetative and sexual reproduction processes is being investigated.

ACKNOWLEDGEMENTS

The authors are particularly grateful to Paul Ashby, PSD, for his advice and also those companies (AgrEvo, Agrichem International Ltd., American Cyanamid and Monsanto) who provided herbicide samples. This work is sponsored by the Pesticide Safety Directorate, an executive agency of MAFF, UK. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

REFERENCES

Campbell P J; Hoy S P (1996). ED points and NOELs: how they are used by UK pesticide regulators. *Ecotoxicology* **5**, 139-144.

Chapman P M; Fairbrother A; Brown D (1998). A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environmental Toxicology and Chemistry* **17**, 99-108.

Sims I; Whitehouse P; Forrow D; Smrchek J (1999). Development and performance charcteristics of a draft OECD *Lemna* growth inhibition test. In: *Abstracts of the 9th Annual Meeting of SETAC-Europe*, 151.

The use and abuse of ready biodegradability tests

W R Jenkins, A Crowe
Huntingdon Life Sciences, Eye Research Centre, Eye, Suffolk IP23 7PX, UK

T Ehrlich
Makhteshim Chemical Works, PO Box 60, Beer-Sheeva 84100, Israel

ABSTRACT

Stringent laboratory tests of ready biodegradability are routinely used for providing the basis for the environmental labelling of new chemicals and for predicting their fate in the natural environment. We reviewed approximately 200 ready biodegradability tests conducted on a wide range of new chemicals in our laboratory between 1990 and 1998 and found that only 13% of those chemicals were readily biodegradable and only 17% would have contributed to risk assessment because the measured level of biodegradation was 60% or more in 28 days. Of the remaining chemicals, 55% were non-degradable under test conditions and 28% showed partial biodegradation. Folpet [(*N*-trichloromethylthio) phthalimide], which failed to achieve the pass level in a standard CO_2 evolution test conducted at 10 mg carbon/L (33 mg Folpet/L, approximately 30 times its water solubility limit), was classified as readily biodegradable when it was re-tested at a level of 1 mg/L (with [U-^{14}C phenyl] material). In was concluded that ready biodegradability tests on poorly soluble chemicals can be unreliable and misleading and are of little value in any risk labelling or assessment scheme.

INTRODUCTION

The determination of the biodegradability of a chemical follows a tiered strategy that is related to its level of production and pattern of use. At the mandatory levels (e.g. the European Union's Reduced Notification and Base Set, Commission Directive 95/36 EC for plant protection products) a screening test is required to determine ready biodegradability and this can be assessed using any one of six internationally accepted methods (Guideline 301, Methods A-F of the OECD). The results of these tests are used in conjunction with information on physicochemical properties and toxicity to aquatic life, to generate data for the classification and labelling of a substance based on its environmental effects (EC Directive 93/21/EEC, 1993), and for assessing the risk it poses to aquatic life (EC Technical Guidance document 1996). The objectives of this study were to review the results of ready biodegradability tests conducted at our laboratory in order to determine the biodegradability "pass" rate and to identify whether categories could be assigned to chemicals that failed the test, based on the observed pattern and levels of biodegradation. In order to determine whether the rate of biodegradation was influenced by the high concentration routinely employed in these tests, we compared the biodegradation of Folpet in a standard CO_2 evolution test at 10 mg carbon/L (33 mg Folpet/L, approximately 30 times its water solubility limit), with the results obtained under similar conditions with [^{14}C]-Folpet ([^{14}C] – phenyl ring, universally labelled) at a test level approximating its limit of solubility (1 mg/L).

MATERIALS AND METHODS

Ready biodegradability tests – analysis of historical data

Tests of ready biodegradability use non-specific analytical techniques to measure removal of Dissolved Organic Carbon (DOC), the consumption of oxygen or the evolution of carbon dioxide during biodegradation. The main conditions employed in the tests, which are of 28-days duration, are summarised in Table 1. Substances are considered to be readily biodegradable in tests employing oxygen uptake or carbon dioxide evolution if the level of biodegradation exceeds 60% of the theoretical value within ten days of the level achieving 10% biodegradation (70% for methods employing DOC removal) – the so called ten-day window criterion.

Table 1. Ready Biodegradability tests and their uses.

OECD method No.	Test	Biodegradability based on:	Not appropriate for:	Test concentration	Inoculum Type
301A	DOC Die away	Removal of DOC	Poorly soluble volatile substances	10 – 40 mg DOC/L	≤ 30 mg SS, ≤ 100 mL sewage effluent
301 B	CO_2 evolution (Modified Sturm test)	CO_2 evolution	Volatile substances	10 – 20 mg carbon/L	≤ 30 mg SS, ≤ 100 mL sewage effluent
301 C	MITI (I) test	O_2 uptake	Some volatiles	100 mg/L	30 mg SS/L
301 D	Closed Bottle test	O_2 uptake	-	2-10 mg/L	≤ 5 mL effluent/L
301 E	Modified OECD screening test	Removal of DOC	Poorly soluble & volatile substances	10 - 40 mg DOC/L	0.5 mLsewage effluent/L
301 F	Manometric Respirometry	O_2 uptake	Some volatiles	100 mg/L, (50 – 100 mg ThOD/L)	≤ 30 mg SS, ≤ 100 mL sewage effluent

DOC – dissolved organic carbon; SS – suspended solids; ThOD – theoretical oxygen demand

The results of 197 ready biodegradability tests conducted in our laboratory between 1990 and 1998 were reviewed in order to determine the "pass" rate and to identify whether categories could be assigned to chemical that failed the tests based on the observed level and pattern of biodegradation.

Determination of the Biodegradability of Folpet in the standard test

The biodegradability of Folpet [(*N*-trichloromethylthio)phthalimide], a foliar fungicide, was determined in a standard CO_2 evolution test at a nominal concentration of 10 mg carbon/L (33 mg Folpet/L, approximately 30 times its water solubility limit).

Folpet (ultrasound-treated suspension, 83.4 mg/100 mL) was added to two vessels containing mineral salts medium inoculated with activated sludge supernatant, to give a nominal test concentration of 10 mgC/L (33 mg Folpet/L). Two control vessels contained inoculated mineral salts medium alone and one contained inoculated mineral salts medium plus sodium benzoate (10 mgC/L). Test and control vessels were aerated and incubated at 20 to 24°C and the CO_2 produced by each culture was trapped in a series of Drechsel bottles containing barium hydroxide. The level of production of $CO_2$was determined by titrating the barium hydroxide with hydrochloric acid using phenolphthalein as indicator.

$^{14}CO_2$ evolution test

In order to determine whether the rate or level of biodegradation was influenced by test concentration, the test was repeated at a later date using a Folpet level of 1 mg/L. [^{14}C]-Folpet ([^{14}C] – phenyl ring, universally labelled; 3 mg; 10% radiolabelled [712.5 kBq], 90% non-radiolabelled) was deposited from a solution in acetone onto the walls of two culture vessels and the solvent was removed in a stream of nitrogen. Mineral salts medium (3 litres) inoculated with activated sludge (30 mg solids/L) was then added to each bottle to give a nominal [^{14}C]-Folpet concentration of 1 mg/L. Control vessels comprised two containing inoculated mineral salts medium alone, and one containing inoculated mineral salts medium plus the reference substance sodium benzoate (10 mgC/L). Test, control and reference mixtures were aerated for 28 days with air that had been treated to remove CO_2. Non-radiolabelled CO_2 produced by control cultures and the culture containing sodium benzoate was determined using the previously described methods. Sodium hydroxide (0.05N) traps were used to collect the $^{14}CO_2$ produced by cultures containing [^{14}C]-Folpet and the levels of $^{14}CO_2$ were measured by liquid scintillation spectrometry (counting). The distribution of radioactivity in the mixtures containing [^{14}C]-Folpet was determined at the end of the test.

RESULTS

Analysis of historical data

The distribution of the study types conducted on a wide range of chemicals in 197 ready biodegradability tests is given in Table 2. The CO_2 evolution test was the most frequently used test because the majority of chemicals tested were poorly soluble and non-volatile.

Table 2. Analysis of biodegradability test types conducted between 1990 and 1998.

Study type	% of total number
CO_2-evolution test	73
Closed Bottle test	17
Manomertic Respirometry	9
DOC removal	1

DOC removal tests were rarely used either because chemicals were not sufficiently water soluble or because loss by adsorption gave poor recovery of DOC in analytical pre-test trials. Manometric Respirometry tests were selectively applied to chemicals that were volatile and the Closed Bottle test, which employs the weakest microbial inoculum, to poorly soluble, volatile or otherwise difficult substances. For water-soluble biodegradable chemicals, the results of ready biodegradability tests are unequivocal and consistent. In ten tests selected from work conducted over a period of approximately two years, the time taken to achieve 60% degradation of the reference substance sodium benzoate in the CO_2 evolution test ranged from 5.3 to 7.1 days and the final levels of degradation ranged from 87 to 94% after 28 days. Of the new chemicals tested, only 13% were classified as readily biodegradable (Table 3) and a total of only 17% showed degradation levels of greater than 60%. Approximately one third showed evidence of biodegradation (>10% but <60%) and over half of the chemicals tested showed little or no biodegradation (<10%) after 28 days so these can be considered to be not degradable under the conditions that were employed in the ready biodegradability tests.

Table 3. Measured biodegradation levels in ready biodegradability tests conducted on a wide range of chemicals.

Classification	% of total number
Pass, biodegradation level >60% in the ten-day window	13
Biodegradation level, 60% in 28 days	4
Biodegradation level >10%,<60%	28
<10% biodegradation	55

Analysis of the results obtained for those new chemicals whose level of biodegradation fell in the range >10% but <60% after 28 days, showed that results fell into two categories:

- a biodegradation plateau was achieved before the end of the test
- a plateau was not achieved and biodegradation was slow but progressive throughout

For those chemicals whose biodegradation appeared to have achieved a plateau in the >10% but <60% category, approximately two-thirds were at a level of <20% and the remaining third, fell in the range >20% but <40%. For those chemicals showing progressive biodegradation, the final levels achieved by the great majority fell in the range >20% <60% degradation which suggests that the pass level may have been achieved if these test had been extended beyond 28-days.

Biodegradation of Folpet at 10 mgC/L and 1 mg/L

The results of the standard ready biodegradability test conducted at 10 mgC/L (33 mg Folpet/L) fell into the previously defined category of chemicals showing the slow but progressive biodegradation that was considered to be typical of those substances whose rate of degradation was limited by their solubility and rate of dissolution. Mean cumulative CO_2 production by the mixtures containing Folpet at 10 mgC/L was equivalent to 41% of the TCO_2 (106.4 $mgCO_2$) at the end of the test (Figure 1). In the later study conducted with ^{14}C-Folpet at 1 mg/L, $^{14}CO_2$ production by mixtures containing Folpet was equivalent to 13% of the applied radioactivity after 4 days of incubation and 63% by Day 14; 73% degradation was achieved by the end of the test on Day 28.

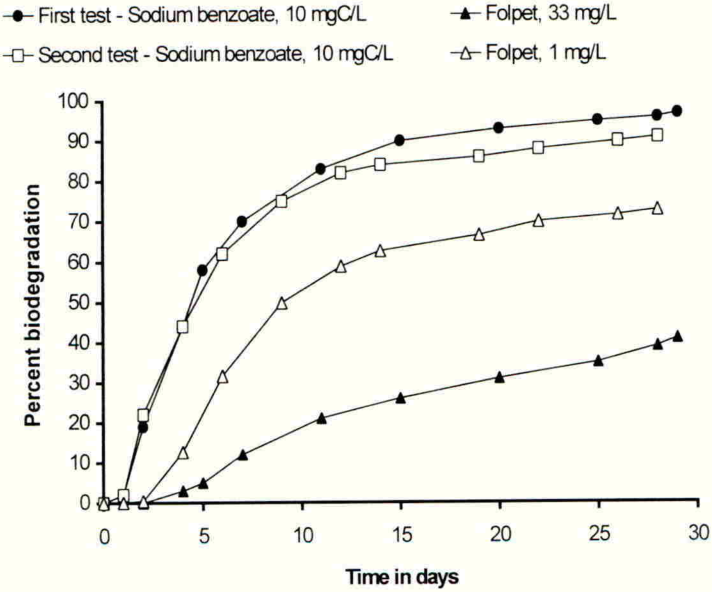


Figure 1. Biodegradation of Folpet at 33 mg/L and 1mg/L

DISCUSSION AND CONCLUSIONS

The majority of information that is currently available for the biodegradability of new and existing chemicals is derived from ready biodegradability tests (Painter, 1992) and very few chemicals have been tested at low concentrations under conditions that simulate those in sewage works or rivers. Because of this, the European Union in their Technical Guidance Document for Risk Assessment only use two classifications of ready biodegradability test results for calculating the predicted environmental concentrations of the water soluble fraction of a chemical after sewage treatment, in surface waters and soil.

- readily biodegradable – achieves the pass level and satisfies the ten-day window criterion.
- biodegradation achieves the pass level in 28 days, but not within the 10-day window

From the results of our analysis, only 13% of the chemicals tested between 1990 and 1998 were readily biodegradable and only 17% chemicals would have been used in risk assessment. For those substances showing partial biodegradation (28% of the total), the results indicate one of two possibilities:

- ***primary biodegradation*** has resulted in the loss of biological activity of the parent molecule indicated by a biodegradation plateau at a level of >10% but <60%.
- ***low solubility and the rate of dissolution of the substance*** may have limited the biodegradation rate (Thomas *et al.*, 1986, Alexander, 1994) and this is quite possible since the majority of the chemicals included in this analysis were poorly soluble.

The results of studies conducted with Folpet suggest that the biodegradation rates of some poorly soluble substances are lower at the high concentrations used in standard ready tests than at levels closer to their solubility limits. Unlike risk labelling based on aquatic toxicity data which is generally based on the measured soluble level of a chemical in a test (UK DOE, 1996), ready biodegradability tests disadvantage poorly soluble substances by calculating the level of biodegradation using the total weight added and not the level in solution. This is a fundamental inconsistency in approach and further research on the biodegradation of poorly soluble substances is urgently needed to allow a more meaningful interpretation of the results of ready biodegradability tests.

REFERENCES

Alexander M (1994). Biodegradation and bioremediation. UK Edition, Academic Press Inc. ISBN 0-12-0409860-X.

Commission Directive 95/36 EC. Amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Annex 1, Section 7. Fate and behaviour in the Environment. Official Journal of the European Communities, **L172**, 22 July 1995.

Department of the Environment (1996). Guidance on the aquatic toxicity testing of difficult substances.

Directive 93/21/EEC, Annex IV: Part II. General classification and labelling requirements for dangerous substances and preparations: Section 5, classification on the basis of environmental effects.

European Commission (1996). Technical Guidance document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances. Part II European Commission Catalogue No. CR-48-96-002-EN-C, ISBN 92-827-8012-0.

OECD Guidelines for Testing of Chemicals, Guideline 301, Ready Biodegradability, adopted 17 July 1992.

Painter H A (1992). Detailed review paper on biodegradability testing. Organisation for Economic Co-operation and Development, Paris 1992.

Thomas, M J; Yordy J R; Amador J A; Alexander M (1986). Rates of dissolution and biodegradation of water-insoluble organic compounds. Applied and Environmental Microbiology, August 1986, 290 – 296.

Effect of two adjuvant types on the distribution of ^{14}C-glyphosate applied to model weed species

S D Sharma, M Singh
University of Florida's Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred Fl-33850, USA

ABSTRACT

Several model weed species, *S. spinosa, A. theophrasti* and *C. obtusifolia*, with varying amounts of polar waxes on their adaxial surface were treated with ^{14}C-glyphosate alone and formulated with X-77 and L-77 to determine the distribution pattern of ^{14}C. It was observed that contact angle and ^{14}C-distribution were significantly affected by both the presence of different waxes on plants and addition of adjuvants in a formulation of glyphosate. The surface tension values of L-77 alone and when added to the herbicide were minimal and so were contact angle values when measured on Teflon slide. Droplets of this solution spread quickly when measured on test leaves. The uptake and translocation of ^{14}C-glyphosate decreased significantly as the percentage of polar waxes increased. However, these values were significantly higher with the addition of L-77 for all test species. This effect may be due to a minimal surface tension, contact angle, and/or solubilization of epicuticular waxes. Greenhouse efficacy evaluation trials with above formulations did not show a similar pattern, although a significantly higher percentage of control of test weed species was recorded when plants were sprayed with glyphosate formulated with an organosilicone.

INTRODUCTION

Glyphosate is a broad spectrum, non-selective and systemic post-emergence (POST) herbicide. It is used extensively for the control of several annual and perennial weeds and is important for a successful weed control programme in citrus groves. It has been reported that different non-ionic surfactants increased cuticular penetration of active ingredient (a.i.) as a result of complex interactions between a.i., surfactant and target species (Stock & Holloway, 1993, Sharma *et al.*, 1996). It has also been reported that organosilicone surfactants enhance the foliar uptake of herbicides into many species because of their ability to spread extensively due to low surface tension, which induces stomatal infiltration (Schönherr & Bukovac 1972). Still, systematic investigations on how modifications to their structure may affect uptake of different a.i. into various plant species are rare. Stevens *et al.* (1991) recorded the relative contributions of the stomatal and cuticular pathways to the uptake of a model polar a.i. in combination with two organosilicone surfactants. They concluded that the ability to access stomatal pathways was exclusive of an ability to enhance cuticular penetration. It has been reported that surfactants may affect spray retention, penetration and may act as humectants (Kirkwood, 1991) and co-solvents (Wyrill & Burnside, 1977).

The plant leaf surface, covered with a lipoidal cuticle acts as a barrier to the penetration of foliage-applied compounds, particularly those having polar characteristics (Holloway, 19982) 1982). The leaf surface may be important in determining the wettability, spray deposition, spread, retention of spray droplets, penetration and may also influence the selection of the proper spray adjuvant (Elmore *et al.*, 1998).

In this study, the weeds teaweed (*Sida spinosa*), velvetleaf (*Abutilon theophrasti*) and sicklepod (*Cassia obtusifolia*), having 14, 64 and 93%, respectively, of polar waxes (Harr *et al.*, 1991) as a percentage of their total waxes on the adaxial surface were used as model test species. To elucidate the effects of two surfactants on penetration, we investigated the effects of both a conventional nonionic surfactant (Ortho X-77) and an organosilicone (Silwet L-77) surfactant on various physicochemical properties and efficacy of glyphosate, and also on the distribution of ^{14}C-glyphosate applied to these model plants.

MATERIALS AND METHODS

Seeds of all three species were sown in 15-cm pots containing a commercial potting compost and were kept in a greenhouse at 25/16°C (±0.5°C) day/night temperature with 70 (±5%) relative humidity. Seedlings were fertilized with 20-20-20 N-P-K fertilizer. In radiolabelling studies, the plants were treated at 3- to 4-leaf stages. For the efficacy study, two seeds of each weed species were planted in 18 holes in a plastic 72 holes tray and were grown until the 4-leaf stage. Plants were sprayed with glyphosate (as Rodeo) @ 0.56 kg a.i./ha both with and without adjuvants in 188 l/ha of water using a Chamber Track Sprayer. The two adjuvants were a nonionic surfactant @ 0.25% v/v and an organosilicone @ 0.1% v/v. Glyphosate as a technical grade salt and ^{14}C-glyphosate with a specific activity of 23.87 mCi/mmol were used in this study. The experimental design was a complete randomized block with four replications in efficacy study and the experiments were repeated twice. Control ratings from 0 to 100% were made at 21 days after treatment (DAT).

For measuring surface tension (ST) and contact angle (CA), a range of dilutions (0.0001 to 0.5%) was freshly prepared for surfactants in distilled water from serial dilution of 1% stock. A range of concentration 0.001 to 0.5% (v/v) of aqueous solutions of surfactant + glyphosate was also prepared. The static surface tension was determined using the DuNouy ring technique by CSC-DuNouy Tensiometer for the different solutions and there were ten replications for each solution tested. The contact angle of 0.001 to 0.5% aqueous solutions of surfactants alone and formulated with glyphosate was measured using NRL Contact Angle Goniometer on Teflon surface (Sharma *et al.*, 1989) and that of 0.05, 0.1 and 0.25% on adaxial surface of plants. For each measurement, ten droplets of 2 μl in size were examined for advancing and receding contact angle.

In ^{14}C distribution studies, all plants were transferred under controlled conditions a week before receiving the treatments. The average photosynthetic photon flux density (PPFD) of 200 $\mu Em^{-2}S^{-1}$ at the plant level; temperatures of 25 and 16 °C (±0.5) and the relative humidity of 55/70 (±5%), day and night, respectively, were maintained. For each plant, the leaf to be treated was carefully covered by aluminum foil and plants were sprayed with the respective treatments using an air pressured Chamber Sprayer delivering 188 l/ha. After removing the aluminum foil, 5x2μl droplets (10 μl) of ^{14}C treatments were applied to a discrete area on the adaxial leaf surface in the median part of 3rd fully expanded leaf at 4-leaf stage. The quantity of ^{14}C applied to leaves was calculated by dispensing a similar number of droplets directly into scintillation vials. Treated plants were dissected into 4 sections (treated leaf, shoot above treated leaf, shoot below treated leaf, and roots) at 72h. The excised treated leaf was washed with 2x4 ml water + ethanol (1:1 by volume) to recover unabsorbed ^{14}C-glyphosate and then rinsed twice with 3 ml of ethanol solution. A 200 μl sub-sample from each washing was dispensed to a vial containing 7 ml of scintillation liquid and then radio-assayed using a Liquid Scintillation Counter (LSC). The plant samples were oven dried at 50°C for 48 h and were combusted using a Biological Oxidizer and quantified by LSC to determine the distribution patterns of ^{14}C-glyphosate. Foliar uptake was defined as [^{14}C] not recovered by washing the treated leaves, and total translocation as the amounts of [^{14}C] which traveled from treated leaf into the rest of the plant parts. Both values were expressed as % of applied dose. Recovery efficiencies of ^{14}C-glyphosate were calculated by adding all the oxidized radioassayed fractions.

The experiment was a complete randomized design with a factorial arrangement of treatments with three replications. The first factor was weed species and second was surfactant. The data were subjected to ANOVA using Pesticide Research Manager software and subjected to Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

The waxes of adaxial leaf surface of 3rd/4th leaf were extracted from three weed species and calculated as described by Bukovac *et al.* (1979). The respective amount of 25.47, 27.6 and 40.61 μg cm^{-2} wax in *S. spinosa, A. theophrasti* and *C. obtusifolia* was recorded.

Effect of surfactant type on the physicochemical properties

Physicochemical properties such as ST, CA and critical micelle concentration (CMC) depend on surfactant chemistry and may be closely related to surfactant type. Inclusion of an adjuvant in the spray solution often reduces the ST, hereby altering the droplet size, distribution of the spray, and increasing wettability and penetration of the pesticide. Our results revealed that the ST values decreased significantly as the concentration increased up to 0.01% for both the surfactants (Figure 1). When surfactants were formulated with glyphosate, significant decrease was recorded up to 0.005%; beyond this point, however, there was no significant reduction in ST (Lownds *et al.*, 1987). This point is known as the CMC (Parr, 1982), which typically occurs at about 0.1 g/L although this varies widely with solution, surfactants and temperature. ST values were significantly lower for L-77 than X-77 when measured either alone or in combination with glyphosate (Sun & Singh, 1998). The improved wettability and penetration mainly results from very complex interactions among the pesticide, adjuvant, carrier water, and the plant surface (Hull *et al.*, 1982; Sharma *et al.*, 1996). A similar trend was also observed when CA was measured on a Teflon slide for either surfactant alone or with incorporation of glyphosate (Figure 2). Contact angle of aqueous

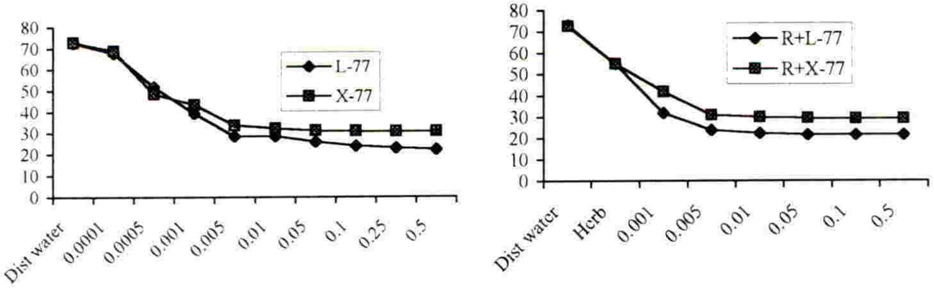


Figure 1. Effect of surfactant concentrations on surface tension of aqueous solutions of (a) surfactant alone and (b) glyphosate formulated with surfactant.

solution of glyphosate alone was significantly higher on *C. obtusifolia* which has 93% polar waxes and much lower on *S. spinosa* having only 14%. When glyphosate was formulated with different concentrations of X-77, the CA was reduced significantly on all the test species. The CA values were significantly lower on *S. spinosa* and *A. theophrasti* than on *C. obtusifolia*. The CA could not be measured for the solution droplets of glyphosate formulated with L-77 as they spread very quickly after application on all the test species (data not presented). It is important to use adjuvant around the CMC with herbicides in order to obtain the maximum effectiveness of herbicidal sprays (Sharma *et al.*, 1989). The beneficial effect of adjuvant concentrations beyond the CMC has been reported by many workers (McWhorter & Barrentine, 1988). But it has been observed in this study and also reported by Abbott *et al.* (1990) that CMC values may not be an especially effective guide to the likely droplet spread of herbicides amended with surfactants.

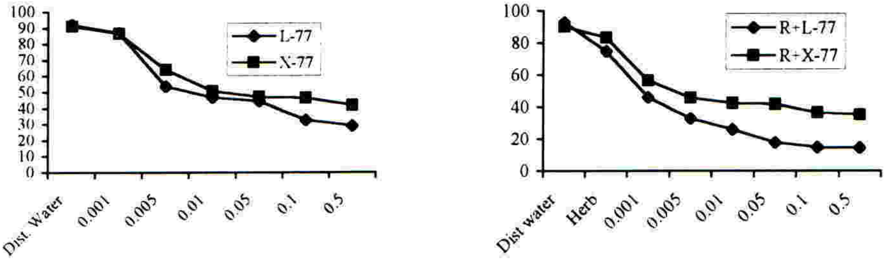


Figure 2. Effect of surfactants on the contact angle of aqueous solutions of (a) surfactant alone (b) glyphosate formulated with surfactants.

ABSORPTION AND TRANSLOCATION OF GLYPHOSATE

All recovered ^{14}C ($\geq$ 95% as % of applied) was assumed to be intact glyphosate, as other researchers have reported little or no metabolism of glyphosate. It was known from an earlier report that both the quantity and quality of waxes present on the leaf surface and the presence of adjuvants in treatments play a major role in the penetration of the a.i. to the cuticle (Elmore *et al.*, 1998). The results of uptake and translocation presented in Table 1 indicate that when ^{14}C–glyphosate without surfactant was applied to the plant surface, significantly higher ^{14}C was absorbed by *S. spinosa* (28.03%) than *A. theophrasti* (21.80%) or *C. obtusifolia* (20.97%). However, with the addition of surfactants, significantly higher uptake of ^{14}C was recorded in all the species. The absorption was significantly higher with L-77 than with X-77 in individual weed species, perhaps due to the very low ST of L-77. Similar results of ^{14}C–glyphosate translocation were obtained with all of the species. The differences in ^{14}C uptake were significant in X-77 formulation but not with L-77 when applied to adaxial surface of *A. theophrasti* and *C. obtusifolia*. However, there was a significant decrease in uptake when the amount of polar waxes increased from 14% in *S. spinosa* to 93% in *C. obtusifolia*. These data confirmed that the lipoidal nature of the surface minimizes the loss of water from the plant and may act as a barrier to the uptake of agrochemicals (Holloway, 1982). Similarly, translocation was significantly lower in *C. obtusifolia* (11.17%) and highest in *S. spinosa* (35.4%) when ^{14}C-glyphosate formulated with L-77 was applied. The excellent surface contact promoted by organosilicone surfactants is a key feature in their mode of action.

Furthermore, it has been suggested that substantially enhanced uptake and translocation may happen because of stomatal infiltration of a.i. (Stevens *et al.* 1991).

Table 1. Distribution of ^{14}C-glyphosate in test weed species.

Treatments	*S. spinosa*	*A. theophrasti*	*C. obtusifolia*
Uptake		% of applied	
Glyphosate (control)	28.03e	21.80f	20.97f
Glyphosate + X-77	58.27b	38.60c	34.23d
Glyphosate + L-77	66.43a	56.87b	56.77b
LSD (P= 0.05)		1.87	
Translocation			
Glyphosate (control)	4.48f	3.78fg	3.40g
Glyphosate + X-77	9.75d	5.87e	6.07e
Glyphosate + L-77	35.4a	20.57b	11.17e
LSD (P= 0.05)		0.80	

The mechanism of surfactant-induced transfer of pesticide a.i. across the cuticle is not established. It could involve one or more of the following processes, as described by Stock & Holloway (1993): i.) change in solubility relationships and partitioning processes that are favorable to transfer, ii.) decrease in the resistance of the cuticle to diffusion, and / or iii.) activation of specific polar or non-polar routes through the cuticle. This study indicated that there was a linear relationship between the physical properties, uptake, and translocation results. However, greenhouse efficacy evaluation trials using these same herbicide/surfactant formulations did not show a similar pattern.

Table 2. Effect of surfactant type on the efficacy of glyphosate on different weed species.

Treatments	*S. spinosa*	*A. theophrasti*	*C. obtusifolia*
		% control	
Glyphosate only (control)	45.0cd	41.3d	43.8cd
Glyphosate + X-77	55.0b	47.5cd	66.3a
Glyphosate + L-77	68.8a	50.0bc	66.3a
LSD (P + 0.05)		6.32	

Significantly higher control of test weed species was recorded when plants were sprayed with glyphosate combined with L-77. The percent control of *S. spinosa* was significantly higher with L-77 (68.8%) than the other two species 21 days after spraying (Table 2). Similarly, Gaskin (1995) reported that many adjuvants do not simply exert their effects on plant surfaces, but penetrate rapidly through the waxy cuticle and into the underlying tissues. The foliar penetration of a surfactant can in turn affect the uptake and translocation of a systemic pesticide, which means that the surfactant providing greatest uptake may not provide greatest efficacy.

REFERENCES

Abbott H A; VanDyk L P; Grobbelaar N (1990). Spreading of spray mixtures on leaf surfaces. I. Relative effectiveness of various physicochemical predictors. *Pesticide Science* **28**, 419-429.

Bukovac M J; Flore J A; Baker E A (1979). Peach leaf surfaces:Changes in wettability, retension, cuticular permeability and epicuticular wax chemistry during expansion with special reference to spray application. *J Amer Soc Hort Sci*, **104**, 611-617.

Elmore C D; Tonos J; Steele M (1998). Epicuticular wax composition of soybean leaves differing in pubescence. *Proc. 5th Intl. Symp. on Adjuvants for Agrochemicals*, ed. P McMullan, Memphis, Tennessee, USA. 49-54.

Gaskin R E (1995). Effect of organosilicone surfactants on the foliar uptake of herbicides: stomatal infiltration *versus* cuticular penetration. *Proc. 4th Intl. Symp. on Adjuvants for Agrochemicals*, ed. R E Gaskin, Melbourne, Australia. 243-248.

Harr J; Guggenheim R; Schulke G; Falk R H (1991). The Leaf Surface of Major Weeds (SANDOZ AGRO Ltd).

Holloway P J (1982). The chemical constitution of plant cutin. In The Plant Cuticle, eds D F Cutler, K L Alvin and C E Price, pp. 45-86. Academic Press: London.

Hull, H M; Davis D G; Stolzenberg G E (1982). Action of adjuvants on plant species. In: Adjuvants for Herbicides. WSSA, Champaign, Illinois. 26-67.

Kirkwood R C (1991). Pathways and mechanisms of uptake of foliage applied herbicides with particular reference to the role of surfactants. In: *Target Sites for Herbicide Acion*, ed. R C Kirkwood, pp. 219-243. Plenum Press: London.

Lownds N K; Leon J H; Bukovac M J (1987). Effects of surfactants on foliar penetration of NAA and NAA-induced ethylene evolution in cowpea (*Vigna unguiculata* L.). *J American Society of Horticultural Science* **112**, 554-560.

McWhorter C G; Barrentine W L (1988). Spreading of paraffinic oil on leaf surfaces of Johnson grass (Sorghum halepense). *Weed Science* **36**, 111-117.

Parr J F (1982). Toxicology of adjuvants, In: *Adjuvants for Herbicides*. Hodgson, R. D. (ed), Monogr.1, WSSA, Champaign, IL. 93.

Sharma S D; Kirkwood R C; Whateley T L (1996). Effect of nonionic nonyl-phenol surfactants on surface physicochemical properties, uptake and distribution of asulam and diflufenican. *Weed Research* **36**, 227-239.

Sharma M P; Singh M; Ling T F; Shah D O (1989). Adjuvant effect of surfactant on herbicidal formulations. In: *Surfactants in solutions*, ed. K L Mittal, pp. 495-508. Pelenum Press: London.

Schönherr J; Bukovac M J (1972). Penetration of stomata by liquids, dependence of surface tension, wettability, and stomatal morphology. *Plant Physiology* **49**, 813-819.

Stevens P J G; Gaskin R E, Hong S O; Zabkiewicz J A (1991). Contribution of stomatal infiltration and cuticular penetration to enhancements of foliar uptake by surfactants. *Pesticide Science* **33**, 371-382.

Stock D; Holloway P J (1993). Possible mechanism of surfactant - induced foliar uptake of agrochemicals. *Pesticide Science* **38**, 165-177.

Sun J; Singh M (1998). Correlation of adjuvant physico-chemical properties and glyphosate efficacy. In: *Pesticide formulations and Application systems*, eds. J D Nalewaja, R Goss and R S Tann. 273-280.

Wyrill J B III; Burnside O C (1977). Absorption, translocation and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. *Weed Science* **24**, 557-566.