Session 1
Processes

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THE MOVEMENT OF WATER AND SOLUTES TO SURFACE AND GROUND WATERS.

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ABSTRACT

The principal mechanisms by which water and solute move in the soil are outlined, emphasizing the link between water flow and solute transport. Preferential flow of water in the macropores of aggregated soils is discussed in relation to its influence on solute transport by convection through the macropores and diffusion out of the micropore region of the aggregates. It is argued that soil and water management can have a significant effect on the loss of solute to surface and ground waters, and evidence from measurements from drained land with different cultivation treatments supports this argument. It is maintained that only a clear understanding of the physics of water movement and solute transport allows effective soil, water and solute management in agricultural systems and the environment.

INTRODUCTION

The movement of water in the soil is the principal mechanism for the transfer of pollutants to surface and ground waters. In the United Kingdom, water draining from the root zone originates mainly from excess winter rainfall although in some areas it may occur as a result of over irrigation in summer months. Water is a solvent capable of carrying significant quantities of dissolved material. In agricultural situations the most important solutes are nutrients, principally nitrogen compounds, and pesticides which can be found in water resources due to runoff and/or leaching from both agricultural and non-agricultural land. The physics of soil water and solute movement can be used to determine the fate of these materials, although the physico-chemical interactions of the solute with soil particle surfaces will vary depending on the nature of the solute as well as the solid surfaces. For example, pesticides usually partition between the solid surface and the water, whereas the nitrate (NO_3^-) anion is associated almost entirely with the water phase of the soil. In this paper the principles of water and solute movement into and through soils is discussed without particular reference to the nature of the solute. An overview of the basic factors controlling leaching is given, and the complications introduced by macropores in soils which give rise to preferential flow, are discussed.

THE PHYSICAL NATURE OF SOIL

Soil is a porous material made up of solid particles which have a size range varying from less than 1μm to greater than 2000μm. The most usual classification of soil is on the basis of particle size distribution (referred to as texture). Soil particles may exist in a variety of packing states, depending partly on their shape and partly on the mechanical stresses to which

the soil has been subjected. These mechanical stresses may arise from natural mechanisms (swelling and shrinking, freezing and thawing, and biological activity within the soil) or through soil manipulation which occurs as part of the soil management activity in farming (operations such as tillage or compaction under tractor wheels). These two factors, texture and packing state, are important for water and solute movement because they determine the pore size distribution in the soil on which flow rates depend.
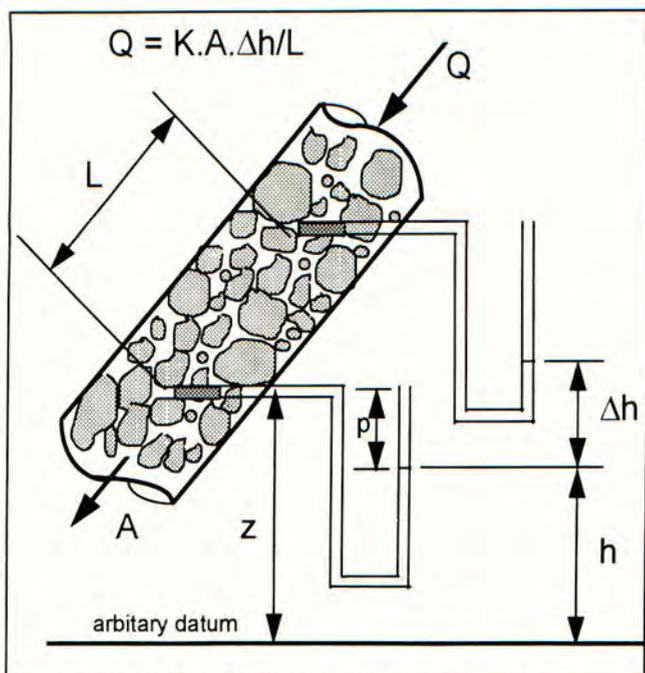
Fig.1 Water flow through soil under an energy gradient, ΔH/L

While considerable attention is often given to soil texture as indicated by the particle sizes within the soil, less attention has been given to pore sizes and their distribution. The soil retains water against the pull of gravity by capillary forces and the attraction of the water to the solid surfaces. Pore water in unsaturated soil is at a lower pressure than atmospheric i.e. at a lower energy level than water at atmospheric pressure, being inversely proportional to the size of the largest undrained pore. This phenomenon is used to characterize soil by measuring the relationship between soil water content and the energy status of the pore water to provide an indication of the pore size distribution. This approach provides only a simple model of what is in reality a very complex geometry but is valuable in that it gives a fair indication of the soil architecture from simple experimental measurements.

SIMPLE DESCRIPTIONS OF WATER MOVEMENT IN THE SOIL

Water flows through the tortuous pathways of the soil pores, interacting with the surfaces of the solid particles which form the boundaries of the pores. Water flow in soil is driven by differences in the energy status of the water, flowing from regions of high energy to ones of lower energy. The principle of water being subject to an energy gradient is the fundamental concept behind all hydraulics and was demonstrated by Darcy in his experimental investigations into water flow down columns of sand (see Freeze, 1994). Fig 1 shows a small column of unsaturated soil consisting of pores and soil grains which is subject to a hydraulic head difference Δh over the length L giving rise to a flow rate Q over its area A. The energy status of soil is measured as the head, h, relative to some datum, and includes both the elevation z from the datum –called the gravitational term– and the pressure head p (positive in ground water but negative in unsaturated soils as indicated by the water levels in manometers that are in equilibrium with the water in the soil pores)–called the pressure term.

Just such measurements were made by Darcy in his columns of saturated sand to derive the well known empirical equation which relates the flow of water through the soil to the energy gradient:

$$v = -K\frac{\partial h}{\partial n}$$

where v is the flow per unit area of soil (Q/A) measured normal to the direction, n, of flow. The proportionality constant K is the hydraulic conductivity for the given soil. For continuity in an unsaturated soil the rate of change of water content must be equal to the flux gradient, so we may write the flow equation as:

$$\frac{\partial \theta}{\partial t} = -\frac{\partial v}{\partial n} = \frac{\partial}{\partial y}\left(K\frac{\partial h}{\partial n}\right)$$

where θ is the soil water content and t is time

In unsaturated soil the hydraulic conductivity K is dependent on the degree of saturation of the soil since empty pores play no part in the liquid flow process. As a soil drains the largest pores drain first and then smaller ones and so on, so that increasingly less and less of the pore space is involved in the flow process. This results in a large reduction in hydraulic conductivity as soil drains. This has important implications for solute movement in unsaturated soils. In most cases flow in the unsaturated zone can be considered to be vertical when water is received uniformly across the soil surface. However there are cases where water movement is two or three dimensional making analysis more difficult. Such cases tend to arise in particular situations such as with trickle and furrow irrigation.

Direction of flow
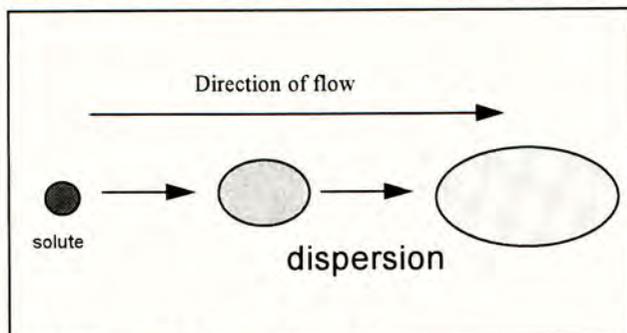
solute

dispersion

Fig 2 Dispersion of a solute in flowing water

It is important to recognize that the approach taken in developing the flow equation, which is widely used in modelling soil water movement, is based on a macroscopic view of the soil. The precise nature of the soil pore geometry need not be known to make predictions of the water flow through the soil. Complications arising from the pore size distribution and the tortuous nature of the pores are accommodated for in the hydraulic conductivity term which has to be found by experiment. The Darcian approach considers only the bulk movement of water. Only by using approximate soil pore space analogies, such as the capillary bundle model, can the flow in particular pores be quantified. The difficulty of estimating individual pore flows with confidence means that little progress in describing water flow in individual pores has been made. However, for single grained soils such as sands, they provide a reasonable description of water flow.

SOLUTE MOVEMENT IN THE SOIL

Solutes dissolved in pore water will be transported by that water when flow occurs. Thus, the simplest mechanism of solute movement in the soil is convective transport with the

flowing water. No solute transport problem can be solved without first considering how the water moves through the soil. The mechanism of convective transport forms the basis of some leaching models.

Water content usually changes down the profile so that mixing of water and solutes results in concentration changes in the soil profile. Thus the transport of solutes is not only by convection with the water but is subject to diffusion and dispersion because of concentration gradients as it moves through the soil profile. This can be demonstrated if we place a drop of dye (a solute) in flowing water. The dye is seen to move in the direction of flow but spreads as it does so, as shown in Fig.2.

The diffusion of solutes along concentration gradients is described by Fick's law that gives the flux F in the n direction as

$$F = D \frac{\partial C}{\partial n}$$

where C is the solute concentration and D is the molecular diffusion coefficient of the solute in the solution in the soil. Longitudinal dispersion occurs in the direction of flow due to flow moving at different velocities in different sized pores under a given head and the tortuous path that the water takes. Lateral dispersion occurs because flow paths are diverging and converging throughout the soil profile. Thus the solute distribution in the soil profile is complicated by lateral and longitudinal concentration gradients and by the velocity distribution in the pores.

The convection dispersion equation combines the convection component and diffusion component and allows for mass conservation of solute. For one dimensional solute movement in the n direction this equation can be written:

$$\frac{\partial C}{\partial t} = v_a \frac{\partial C}{\partial n} + D_a \frac{\partial^2 C}{\partial n^2}$$

Here the first term on the right hand side of the equation uses the average pore water velocity, v_a, to describe the convective transport in the soil. It should be noted that the use of the average pore water velocity in this way requires a uni-modal normal pore size distribution. The second term on the right hand side combines both the diffusion of solutes in the soil water and the dispersion of solute that occurs due to the spreading of the solute within the profile as a result of different velocities of flow in different sized pores. The term D_a is termed the hydrodynamic dispersion coefficient and has the same dimensions as the diffusion coefficient but depends on the pore water velocity. When the velocity of water is zero, the hydrodynamic dispersion coefficient reduces to the diffusion coefficient.

The pore water velocity, v_a, term is assumed to be equal to the Darcy velocity divided by the water content of the soil (v / θ), that is the average flow velocity in the pore space. Thus, use of both the flow equation and the convection-dispersion equation allows a description of solute transport down soil profiles. In particular, good agreement between predictions of solute breakthrough and distribution is found for flows in sand columns in the laboratory under simple steady state situations.

The consequence of hydrodynamic dispersion is illustrated in Fig.3 which shows how a solute initially at the soil surface is transported downwards with the flow through a saturated

soil profile to give the characteristic distribution shown by the curve (a). Here some solute remains in the smallest pores near the soil surface and some travels to depth in the largest pores. The bulk is found at some intermediate depth. Field observations show similar concentration profiles during leaching. Where the solute is partitioned between the solid and liquid and decays with time, its movement is restricted and the distribution shown in curve (b) in Fig.3 is observed.
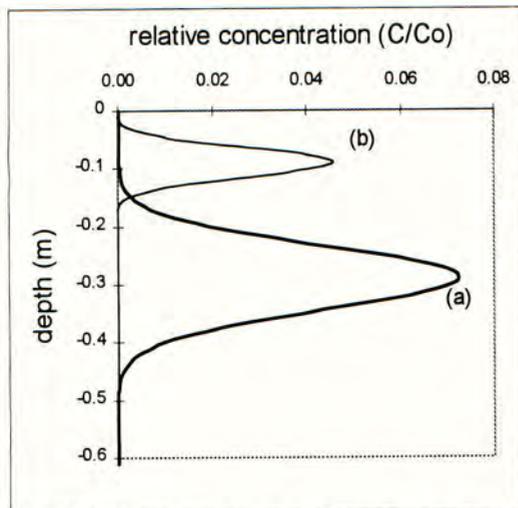
Fig. 3 Solute concentration profiles some time after the start of leaching for (a) a non-reactive solute and (b) a reactive decaying solute.

In practice the use of this hydrodynamic dispersion approach is complicated by the need to parameterise the equations and to be able to define precisely the boundary conditions imposed on the system. However, more significantly, the equation has been formulated and tested for simple porous materials such as sands as used by Darcy in his experiments. Complicating factors, such as soil heterogeneity, soil swelling and shrinking, and soil aggregation, can vitiate predictions made by this macroscopic approach. In particular, use of a mean pore water velocity in aggregated soils with a bi-modal pore size distribution does not give a good description of the solute movement in such soils.

FLOW IN AGGREGATED SOILS

Where macropores occur in soils, considerable complications are introduced if we try to describe the flow of water and solutes using the 'sand physics' approach described above. In well aggregated soil the aggregates have pore sizes which are many orders of magnitude smaller than the macropores (see Fig.4). In these cases, although the macropores may represent a very small fraction of the total porosity, water movement in wet soil can be predominantly in these large pores, making the saturated hydraulic conductivity of the bulk soil very much greater than that of the micropore region formed by the soil aggregates, the measurement of the hydraulic properties of which is possible (Leeds-Harrison *et al.*, 1994). The opposite is true however in an unsaturated soil where the conductivity of the bulk soil is often very much less than that of

Fig.4 Solute diffusion form aggregates into water flowing in the macropores.

the individual aggregates because the empty macropores act as barriers to flow. A comparison of bulk and aggregate saturated hydraulic conductivity for a tilled clay soil at Silsoe, Bedford, gave values of 1.0 m/d and 0.012 m/d respectively. As with single grained soil the packing of the aggregates will have a considerable effect on the bulk soil conductivity so that we must expect soil management to play an important role in determining the behaviour of water and solutes in such soils. This effect is most notable in clay soils in which pore sizes in the clay aggregates are of the order of a few microns and the macropores may be 1 or 2 mm in size.

Macropore flow occurs not only in wet soils but also in dry ones when there is intense rainfall. Water arriving at the soil surface may exceed the intake rate of the aggregate in the same way as for infiltration into a structureless soil. In the case of the aggregated soil the water runs off the aggregate and then flows into a macropore. Thus, the initiation of macropore flow is determined by the infiltration properties of the aggregate material and the rate at which the water is received at the soil surface. Because the intake rate of wet aggregates is less than that of dry ones, macropore flow is more likely in wet soil conditions. Jarvis and Leeds-Harrison (1987) have used this physical consideration to describe the movement of water in a cracking clay soil. It should not be thought, however, that the preferential movement of water in macropores is confined solely to heavy clay soils although they represent probably the worst (and most difficult) cases. In a study of fourteen different soil textural types in Switzerland, Flury *et al.* (1994) using a dye technique found that twelve exhibited distinct macropore flow behaviour. In addition they show that the depth of penetration of water containing a dye was greater under more intense irrigation.

Fig.5 Solute concentration profile some time after the start of leaching in a macro porous soil.

Solute movement in aggregated soils is complicated by the preferential flow in the macropores which may transport solutes rapidly through the root zone. However aggregates may also have solutes distributed through them; for instance solutes may diffuse into the aggregates or be transported into them as the soil wets up. A fuller description of the processes of water and solute exchange between macropores and the micropore region of the aggregates is given by Youngs and Leeds-Harrison (1990). Within the aggregate we may apply the same physics to water and solute movement as has been described above for simple porous materials. However in many aggregates the velocity of water movement is very slow and the dominant mechanism of solute transport out of the aggregates when surrounded by macropores filled with water at a lower concentration, is diffusion. Within the macropore it is likely to be convective transport that dominates the transport process. These two processes occur simultaneously in wet soils so that water flowing in the macropores picks up solute as it

diffuses from the aggregates. Thus, a solute initially at the soil surface is rapidly transported to depth by this combination of mechanisms and the solute concentration profile illustrated in Fig.5, is markedly different from that for the sand column in Fig.3. Where solute is initially evenly distributed through the profile there is likely to be an increasing amount of solute in the macropore water as it is transported downwards. This means that the concentration gradient between the aggregate and the macropore water decreases with depth so more leaching occurs in the upper layers than lower down the profile.
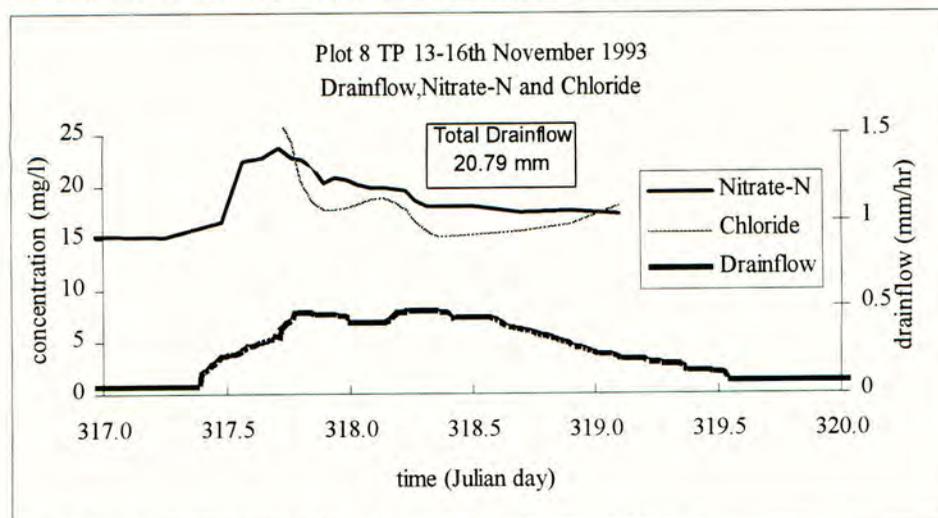
Fig.6 Hydrograph and chemograph for a ploughed (inversion tillage) clay soil
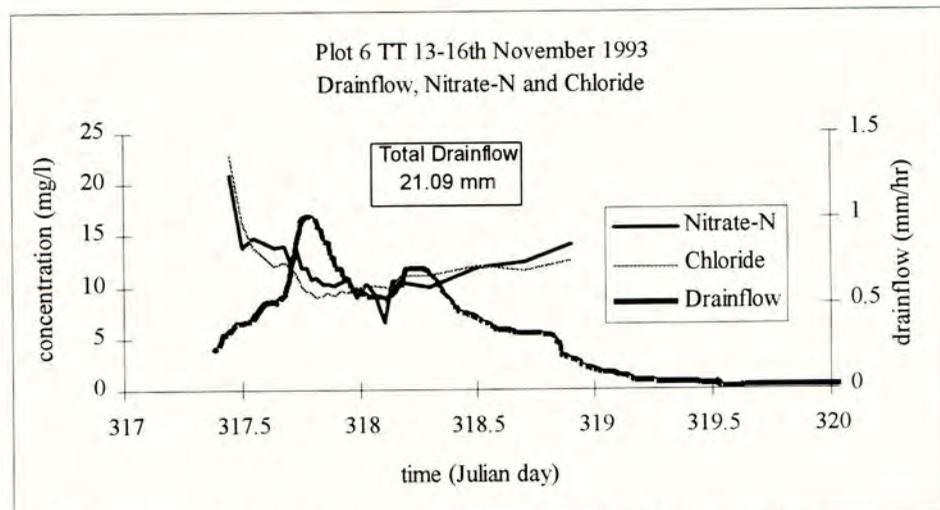
Fig. 7 Hydrograph and chemographs for a vertically tined (non inversion tillage) clay soil

It can be shown that aggregate size has a great effect on the rate of release of solute under these conditions. Changes in soil water and solute concentration within the aggregate occur on a time scale inversely proportional to the square of its size. Thus, tillage and soil management, which influence aggregate size, will be expected to have an effect not only on soil hydrology but also on the leaching behaviour of the soil. Figs. 6 and 7 show data for

nitrate and chloride leaching from a drained clay soil under different primary tillage management. In the case of conventional inversion ploughing (Fig 6) we see that the concentration of nitrate rises as the drain flow proceeds whereas the concentration of chloride falls. In the case of the vertically tined soil, a non-inversion tillage treatment, both nitrate and chloride concentrations fall as the drainflow rises to a peak as shown in Fig.7. This phenomena may be explained by the nature of the flow paths and the sizes of the soil aggregates. Nitrate may be assumed to have originated from the top layer of soil which is well enough aerated for nitrification processes to occur. The aggregates in the ploughed soil are generally smaller than those in the vertically tined soil, but ploughing has disrupted the continuity of the vertical macropores at the base of the plough layer and so restricts the downward movement of water to the drains. In this case the transport of the solute is limited by the rate at which the water is moving through the soil. Diffusion from the small aggregates is fast enough for the water to increase its concentration as the flow proceeds. In the case of the vertical tine treatment the water travels rapidly to the drain, as shown by the peakier hydrograph, and nitrate transport is limited by the rate at which it can diffuse from the larger aggregates which are formed with this soil management practice.

The chloride originates in the profile as a result of deposition with rainfall and accumulates throughout the soil profile in the summer months. It is found at greater depths in the soil profile than the nitrate. Thus, in both cultivation treatments it tends to be associated with larger aggregates, and thus the flow limiting situation is seen in both treatments with this solute. This behaviour of water and solute in these cases is determined therefore by soil management and shows the danger of assuming a particular transport behaviour based solely on soil texture considerations. The phenomenon of flux limited transport may occur for other chemicals; for example, preliminary data presented by Johnson et al. (1993) appears to show a similar flux limiting case in their studies of pesticide movement to drainage waters in a clay soil in Oxfordshire.

Addiscott and Dexter (1994) present a simplified description of soil architecture which consists of a regular arrangement of cubes (the 'sugar cube model'). When this is disturbed,
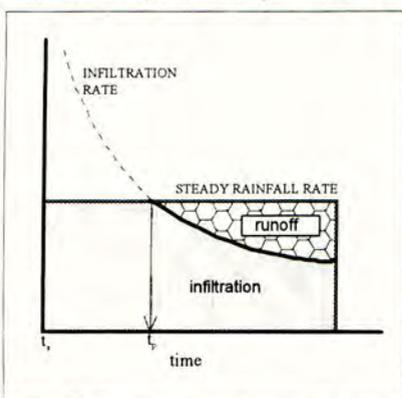
Fig. 8 Infiltration rate during rainfall at a constant rate.

for instance as may occur with tillage, the soil is rearranged into a much more complex porous material in which the cubes themselves retain their original properties but the macropore space is considerably changed. Dexter (1993) has demonstrated that at the particle scale water flow at the base of a bed of gravel can be extremely heterogeneous and Bouma and Dekker (1979) have shown that only a small proportion (2-4%) of the surfaces of aggregates are wetted during macropore flow. It is clear therefore that soil structure created by different management systems will have a significant effect on the distribution of flow within the soil profile and the interaction of the macropore water with that in the aggregate. Much still needs to done to better describe the impact of soil structure on solute transport. Control over the fineness of tilth and macroporosity are areas where research is still needed.

INFILTRATION

Water arriving at the soil surface as rainfall or irrigation will infiltrate if the soil's potential uptake rate is greater than the rate at which the water is supplied. Energy gradients determine the rate and direction of flow. As soil wets its uptake rate declines as the hydraulic gradient gets smaller, and there may come a point in a storm event when the received rate is greater than the uptake rate and ponding that can give rise to runoff occurs. However, even during runoff water continues to infiltrate into the soil even though the soil is saturated at the surface.

As illustrated in Fig 8 for a constant rainfall rate the infiltration rate is constant with time from the start (t_s) until ponding occurs at some time (t_p). The quantity of rainfall that goes to surface runoff is shown by the shaded portion on the graph, the remainder infiltrates. We may observe this phenomena on all soils although because different soils have different infiltration characteristics, determined principally by the size of the pores at the soil surface, the time to ponding and the partition between runoff and infiltration and the quantities of transported solutes will vary according to soil type and rainfall intensities.

DRAINAGE

So far the discussion of the movement of water and solute has implied only vertical movement to the water resource. This is reasonable where the water draining from the upper soil horizons continues to depth in unsaturated soil down the profile, eventually recharging a deep aquifer. In less permeable soils, however, a water table usually develops close to the soil surface and drainage systems must be installed to prevent the soil profile becoming saturated. In this case water flows almost vertically in the unsaturated zone (Childs 1945) but diverges from the vertical and becomes more horizontal in the saturated zone. Where drains are installed in the field the water table is curved being highest mid-way between the drains on level ground. Widely spaced drains lead to the soil becoming more saturated than with closely spaced drains, and the retention time of water in the soil may be controlled by the spacing at which drains are installed.

Recession times for drainage are determined not only by the drain spacing but also by the hydraulic conductivity and drainable porosity of the soil. These are affected by soil management. The hydrographs shown in Figs. 6 and 7 reflect the differences in soil hydrograph response between ploughing and vertical tine non-inversion tillage. Recent analysis (Dougherty et al. 1994) of the recession time constant for wheeled and un-wheeled plots on a tilled clay soil shows that wheeled plots have a significantly longer recession time. This is important for solute movement as this means longer retention of water in the soil profile and possible equilibration of macro-pore water with solutes in the matrix.

CONCLUSIONS

In this discussion of water and solute movement in the soil we have concentrated on transport in wet soils. We know that this is the condition in which leaching occurs and so it is right that the discussion has emphasized this aspect of the processes. However it should not be forgotten that water and solute can move in drier soils so that some redistribution can

occur in such conditions. Water velocities are usually much less under dry conditions than in the wet conditions and thus, where we are concerned with the appearance of solutes in water resources, consideration of episodic wetting events is appropriate.

Nothing can be done about the variable nature of weather and climate but a clear understanding of the basic principles of the leaching problem should allow us to better manage our agriculture and environment. The physics of the movement of water and the transport of solutes in soils presented here is often used as the basis of models. Analytical solutions to the transport equations may be found for some simple boundary problems in leaching situations, but more commonly numerical solutions are used. This in itself can introduce complications, because 'modellers' are then faced with a variety of techniques for dealing with difficult boundary condition problems. In both analytical and numerical solutions the precision with which soil parameter values can be identified may override this physics based approach and simpler models may be employed. Only by understanding the basic principles of water and solute flow can the efficacy of models be judged.

It is hoped that this short paper has at least raised the issues of importance so that the problem of leaching can be approached in a mechanistic way. It must be stressed that justice to the science cannot be achieved in a short paper and that significant issues of scaling these principles from the single vertical profile to the catchment scale still present the hydrologist with a significant challenge.

REFERENCES

Addiscott, T.A. and A.R. Dexter (1994) Tillage and crop residue management effects on losses of chemicals from soils *Soil & Tillage Research* **30**,125-168

ouma, J. and L.W. Dekker (1978) A case study on infiltration into dry clay soil. I. Morphological observations *Geoderma* **20**, 27-40

Childs, E.C. (1945) The water table, equipotentials and streamlines in drained land. III *Soil Sci.* **59**, 405-415

Dexter, A.R. (1993) Heterogeneity of unsaturated, gravitational flow of water through beds of large particles *Water Resources Research* **29**, 1859-1862

Dougherty, E, P.B.Leeds-Harrison and E.G.Youngs (1994) The influence of soil management on drainage hydrographs. *Soil Use and Management* (submitted)

Flury, M., H. Fluhler, W.A. Jury and J. Leuenberger (1994) Susceptibility of soils to preferential flow of water: a field study *Water Resources Research* **30**,1945-1954

Freeze, R.A. (1994) Henry Darcy and the fountains of Dijon. *Groundwater* **32**, 23-30

Jarvis, N.J. and P.B.Leeds-Harrison (1987) Modelling water movement in drained clay soil. I: Description of the model *Journal of Soil Science* **38**, 487-498

Johnson, A.C, A. Haria, C. Batchelor, J.P. Bell, and R.J. Williams (1993) Fate and behaviour of pesticides in structured clay soil: Institute of Hydrology, Wallingford.

Leeds-Harrison P.B., E.G.Youngs and B Uddin (1994) A device for determining the sorptivity of soil aggregates *European Journal of Soil science* **45**, 269-273

Youngs, E.G. and P.B. Leeds-Harrison (1990) Aspects of transport processes in aggregated Soils *Journal of Soil Science* **41**, 665-675

STUDIES OF TIME-DEPENDENT SORPTION PROCESSES IN SOILS

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ABSTRACT

Results from laboratory experiments with a range of soil acting herbicides indicate an increase in the strength of adsorption with increasing residence time in the soil. This was particularly marked with metsulfuron-methyl. Similar results were obtained with alachlor in columns of soil in the field where water available residues in the surface soil layers declined much more rapidly than solvent extractable residues. Investigations of rates of adsorption of isoproturon indicated slower adsorption in static compared with shaken experimental systems with the differences more pronounced in a highly structured clay soil than in an unstructured sandy loam. The implications of the results for modelling of pesticide transport are discussed.

INTRODUCTION

One of the main properties of a pesticide that controls its movement in soil is its partition coefficient between soil surfaces (principally clay and organic matter) and the soil solution. This is usually expressed as an adsorption distribution coefficient (Kd) or as an average partition coefficient between soil organic carbon and the soil solution (Koc). As discussed previously (Walker, 1987) accurate description of the distribution of chemicals between the adsorbed and solution phases is an essential component of any mathematical model of pesticide leaching since only material in free solution will be readily mobile. Most models assume complete equilibration of adsorption within any soil layer, and they assume that adsorption distribution coefficients are constant for any soil-pesticide combination. Adsorption measurements are also usually made with sieved and air-dried soils in well equilibrated laboratory experiments. In the field during heavy rainfall events, water will move through moist, natural soil, and may move rapidly downwards. The extent of adsorption by moist soils with short contact times may therefore be of some significance. During any leaching event, the process of desorption will also be important, particularly in the surface soil layers. Rainfall entering the soil will displace water downwards and rewet the soil, thus changing the equilibrium between adsorbed and solution phases. There is evidence that desorption becomes more difficult with increasing residence time of a pesticide in the soil. Boesten & Van der Pas (1983) demonstrated that the apparent desorption distribution coefficients for cyanazine and metribuzin measured after 56 days incubation in soil were two to three times greater than those measured initially. Similar results have been reported for picloram (McCall & Agin, 1985), linuron, isoxaben and propyzamide (Walker, 1987), and isoproturon (Blair *et al*, 1990). The objective of the present paper is to describe some alternative techniques that can be used to measure pesticide adsorption/solution relationships, and to provide further information concerning time-dependent sorption processes in soils.

EXPERIMENTAL METHODS AND RESULTS

Changes with time in aqueous availability of linuron, isoxaben and propyzamide residues

Walker (1987) incubated linuron, isoxaben and propyzamide in samples of a sandy loam soil (1.2% organic carbon, 17% clay, 77% sand, pH 6.7) at 20°C and with soil moisture at a potential of -33 kPa. At intervals over a period of 47 days, subsamples were removed from each treatment and measurements were made of total (solvent) extractable residues and of aqueous extractable residues. The water available residues were measured after shaking on an end-over-end shaker for 24 hours. The data were expressed as desorption distribution coefficients (Kdes) and they are shown graphically in Figure 1. The results in Figure 1a demonstrate an increase in desorption distribution coefficient with time for all three herbicides, with the values at 47 days almost twice those recorded immediately after mixing the chemicals into the soil. The data are replotted in Figure 1b with the distribution coefficients expressed as a function of the square root of time. An approximate linear relationship was obtained for all three compounds, suggesting the possibility of a diffusion controlled process.

Behaviour of metsulfuron-methyl

Experiments similar to those described by Walker (1987) have been made recently with the sulphonylurea herbicide metsulfuron-methyl. Adsorption distribution coefficients for this compound are generally very low when measured using conventional shaking techniques (Walker *et al.*, 1989) but significant amounts of the herbicide are retained in surface soil layers in the field (Walker & Welch, 1989). In the current experiments, metsulfuron-methyl was incorporated into samples of soil from two experimental areas (Hunts Mill and Cottage Field) at Horticulture Research International using the techniques described in detail previously (Walker, 1987). The Hunts Mill soil was a sandy loam with 1.37% organic carbon and pH of 6.5, and the soil from Cottage field was a relatively well structured clay loam with 2.58% organic carbon and a pH of 6.8. The initial concentration of metsulfuron-methyl was 250 μg/kg dry soil and the herbicide dose comprised both formulated and carbon-14 labelled chemical. The initial concentration of radioactivity was 300 Bq/g dry soil. The soils were incubated at 20°C with soil moisture at a potential of -33kPa. At intervals over a 49-day period, subsamples of soil were removed (25 g) and the herbicide extracted with either methanol plus water (80 + 20) containing 0.5% acetic acid (Walker *et al.*, 1989) or with 0.02M calcium chloride solution (25 ml) by shaking for 2 hours on a wrist-action shaker. The samples were allowed to stand until the soil had settled, when duplicate subsamples of the supernatant were mixed with Optisorb-HiSafe scintillation cocktail and counted using a RackBeta liquid scintillation counter with external standard quench corrections. Occasional samples were checked for the presence of degradation products using the tlc method described by Smith (1986) which demonstrated that the extracted radioactivity always comprised at least 95% parent metsulfuron-methyl.

The results in Figure 2a demonstrate a marked difference in rate of degradation of the herbicide between the two soils. In the Hunts Mill soil, approximately 50% of the initial dose remained after 49 days, whereas in the soil from Cottage field, the time to 50% loss was approximately 15 days. The relative amounts of total extractable and aqueous extractable herbicide permit calculation of apparent desorption distribution coefficients (Kdes) and these are shown in Figure 2b. The data show a marked increase in distribution
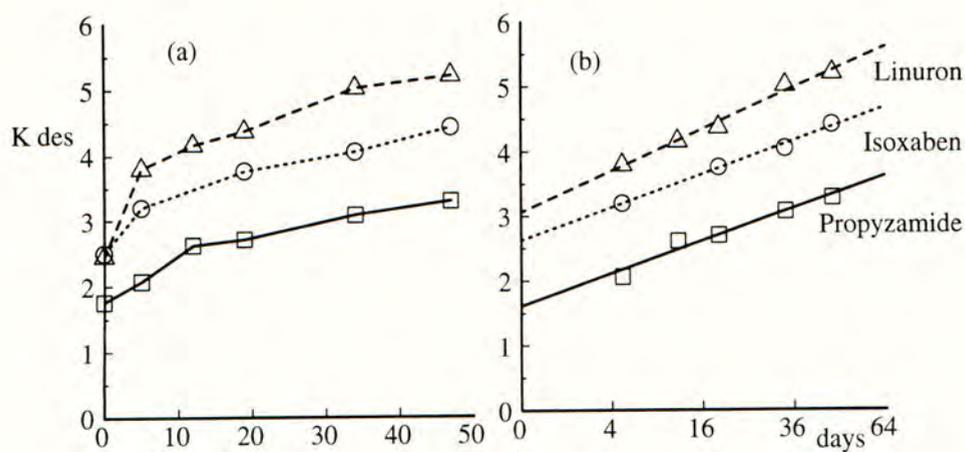
Figure 1. Changes with time in desorption distribution coefficients
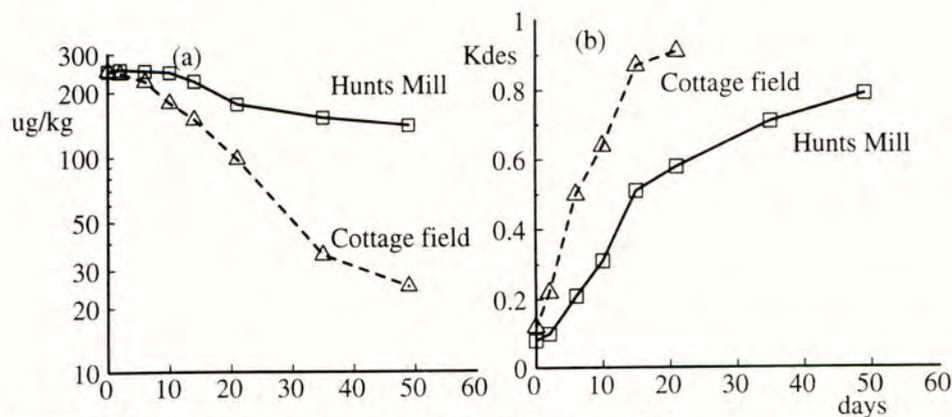
Figure 2. Degradation and desorption of metsulfuron-methyl
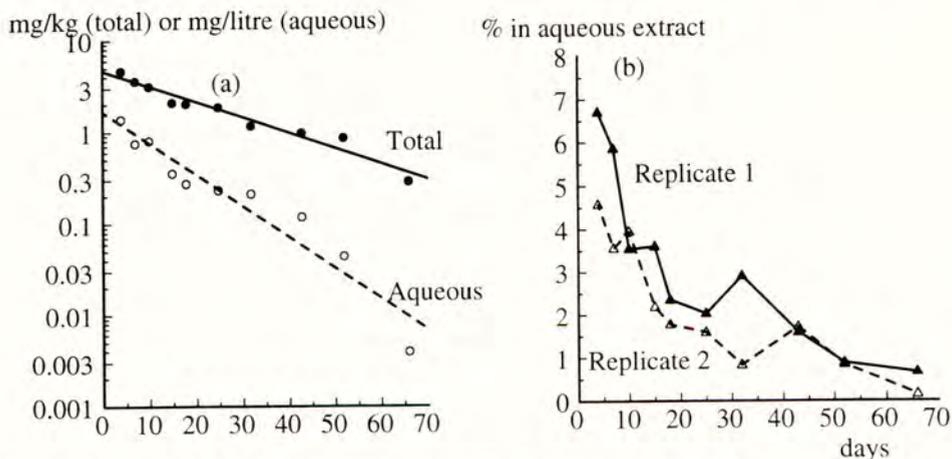
Figure 3. Changes in total and aqueous extractable residues of alachlor

coefficient with increasing residence time in the soil.

Behaviour of alachlor in soil columns in the field

Further observations of apparent changes in strength of adsorption were made with the herbicide alachlor in an outdoor experiment. The soil was a clay loam containing 2.1% organic carbon and with pH 6.9. Four rigid polypropylene columns (20 cm diameter x 20 cm) were inserted into soil in the field immediately prior to application of alachlor at 2.5 kg a.i./ha. After spraying, the columns were removed and the bottom of each held in place by 0.25 mm mesh nylon gauze fixed to the tube with waterproof sealing tape. A rigid polypropylene funnel (20 cm diameter) was sealed to the bottom of each column. The columns were then mounted in a mini-lysimeter system (Moon & Walker, 1991). At intervals of 3 to 7 days over a 70 day period 5 samples of soil were removed from the surface of each column using a cork borer (1 cm diameter; 2 cm long). Two replicate groups of cores were placed in conical flasks (25 ml) and extracted with acetone; the other two replicate groups of cores were placed in the body of a 10-ml disposable syringe the outlet of which was plugged with glass wool. Hplc vials were attached to the bottom of the syringe bodies and distilled water (1 ml) added to the soil. After 30 minutes, the samples were centrifuged and the extracted water was collected in the HPLC vials. The resulting solution was analysed by glc (Moon & Walker, 1991). The results (Figure 3) demonstrate a more rapid decline in aqueous extractable than in total extractable residues.

Rates of adsorption of isoproturon by fresh and air-dried soils

The three experiments described above investigated the changes in aqueous availability of residues with increased residence time in the soil and therefore provide information concerning ease of desorption. Another potentially time-dependent step in the leaching process is the rate at which a pesticide equilibrates with soil during water flow, i.e. the rate of adsorption. An experiment was made with two contrasting soils to investigate the influence of experimental variables on the rate of equilibration of isoproturon with soil. The soils used were the unstructured Hunts Mill sandy loam soil described above, and a highly structured clay soil from Wytham, Oxfordshire (organic carbon, 2.57%; clay, 41.5%). Samples of the soils were collected from the field and either air-dried and sieved (2-mm mesh), or kept in their natural, fresh state. The moisture contents of the samples were determined by drying overnight at 105°C. Subsamples of the soils (10 g dry weight) were weighed into conical flasks and solutions of isoproturon (10 ml, 1mg l^{-1}, analytical grade plus carbon-14 labelled, 150 Bq ml^{-1}) were added. Half of the samples were shaken continuously on a wrist-action shaker. The other half were swirled gently for about 10 seconds and then left to stand on the laboratory bench. At intervals of 5, 10, 30, 60, 120, 240 and 480 minutes, duplicate subsamples from all of the treatments were centrifuged and duplicate amounts (1 ml) of the clear supernatant were counted as described above. Adsorption was calculated from the difference between initial and residual radioactivity. The results expressed as distribution coefficients (Kd) are shown in Figure 4. They indicate stronger adsorption in the Wytham than in the Hunts Mill soil which is consistent with the differences in their organic carbon and clay contents. There were also marked differences in adsorption distribution coefficient with time and between treatments within any one soil. In the Hunts Mill soil, distribution coefficients at 480 minutes were 1.5 to 2-fold greater than after 5 minutes and were marginally greater in the shaken than the static samples. There appeared to be little effect from air drying and sieving of this soil. In contrast, the
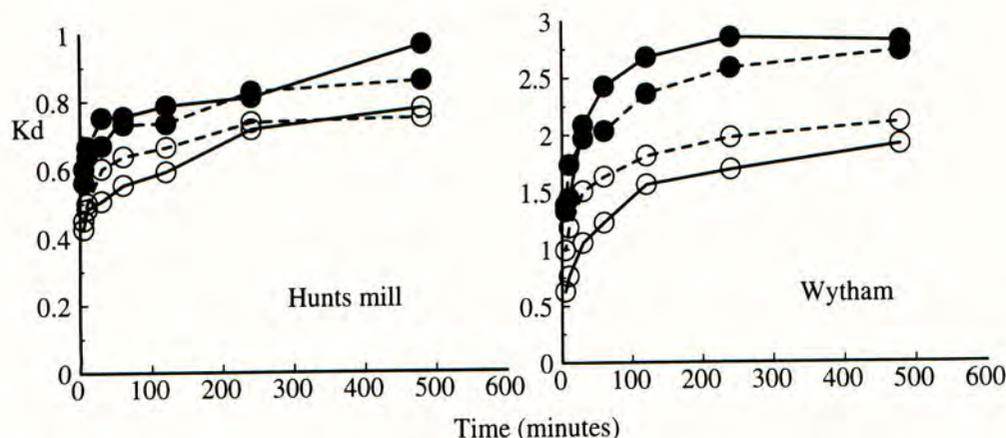
Figure 4. Rates of isoproturon adsorption by two soils initially fresh —— or air dried - - -, using a shaken ● or static ○ system

results from the Wytham soil indicate 2 to 3.5-fold increase between 5 and 480 minutes, a large decrease in adsorption in static compared with shaken systems, and a distinct difference between the results for the air-dried and for the fresh soil samples

DISCUSSION

A general observation in field monitoring exercises is that the largest concentrations of pesticide in drainage water occur following the first major rainfall event after application and decline thereafter (Jones, 1993). This is expected, in part, because of degradation of the pesticide over time leading to lower concentrations in the soil. The present results indicate that an additional contributary factor may be a change in partition coefficient with increasing residence time so that a smaller proportion of the total residue remains available for movement as time progresses. The results with linuron, isoxaben and propyzamide (Figure 1) suggest a 1.5 to 2-fold increase in distribution coefficient over a period of 48 days, and those for metsulfuron-methyl (Figure 2) indicate an 8 to 10 fold increase over a similar time period. Recent results of Duffy *et al* (1993) provide evidence that many sulfonylurea herbicides (including metsulfuron-methyl) are degraded primarily in the water phase of the soil, and that restricted rates of release from the adsorbed phase lead to reduced rates of degradation as time progresses. The present results are consistent with this hypothesis, since the apparent increase in partition coefficient of metsulfuron-methyl was more rapid in the soil showing the most rapid rate of loss. There was also a suggestion that rates of degradation in both soils were somewhat slower with increasing residence time.

The above results were produced in controlled laboratory experiments following uniform incorporation of the chemicals into the soil. The major dissipation process under these conditions will be degradation. In the field, other processes will be important. Readily available pesticide will be removed from the surface soil layers by leaching during rainfall events, and hence if the rate of desorption is slow, this will exaggerate the changes in aqueous available residues over time. The results in Figure 3 suggest that this does occur with alachlor, with only small proportions of the total residues readily removed by water after 65-70 days. Between 4.5 and 6.5% of the total residue remaining was

extractable with water shortly after application, but this decreased to less than 0.5% of the total residue remaining after 66 days. The results in Figure 3b show a trend very similar to that observed in drainage water concentrations over time (Jones, 1993).

An additional factor that may be significant in terms of explaining deep movement of trace levels of pesticides in soil is the rate at which dissolved chemical will re-equilibrate with the soil during downwards transfer. When the intensity of rainfall is low, the rate of water movement will also be low, and there may be adequate contact time between the pesticide and the adsorbing surfaces. When rainfall intensity is high, these contact times may be short, particularly if a significant proportion of the water flows through cracks or fissures and bypasses the main soil matrix. The results in Figure 4 indicate that the adsorption distribution coefficient for isoproturon in a highly structured soil with just 5 to 10 minutes contact may be less than 30% of that observed after 4 to 8 hours. The most significant feature of the results in Figure 4 is that the adsorption distribution coefficients were always smaller when measured in a non-shaken compared with shaken systems.

The present results indicate that use of simple equilibrium adsorption distribution coefficients in mathematical models of pesticide mobility may give incorrect predictions of movement. Desorption distribution coefficients may well be considerably higher than adsorption coefficients, particularly at increased times after application. In contrast, adsorption coefficients during rapid water flow might be less than those measured using standard equilibrium techniques. The combined effect of these phenomena could well result in greater than expected residues near the soil surface and marked tailing of distributions deeper than expected in the soil profile. The results indicate that a correct interpretation of adsorption-desorption data is essential to accurate prediction of pesticide movement.

REFERENCES

Blair, A.M.; Martin, T.D.; Walker, A.; Welch, S.J. (1990) Measurement and prediction of isoproturon movement and persistence in three soils. *Crop Protection,* **9**, 289-294.

Boesten, J.J.T.I.; Van der Pas, L.J.T. (1983) Test of some aspects of a model for the adsorption/desorption of herbicides in soil. *Aspects of Applied Biology,* **4**, 495-501.

Duffy, M.J.; Carski, T.H.; Hanafey, M.K. (1993) Conceptually and experimentally coupling herbicide sorption and degradation in soil. *Proceedings 9th Symposium on Pesticide Chemistry,* Piacenza, Italy, 295-308.

Jones, R.L. (1993) Role of field studies in assessing environmental behaviour of herbicides. *Proc 1993 Brighton Crop Protection Conference - Weeds,* 1275-1282.

McCall, P.J.; Agin, G.L. (1985) Desorption kinetics of picloram as affected by residence time in soil. *Environmental Science and Technology,* **4**, 37-44.

Moon, Y-H.; Walker, A. (1991) The degradation and mobility of alachlor in a sandy loam soil. *Proceedings 1991 Brighton Crop Protection Conference - Weeds,* 499-506.

Smith, A.E. (1986) Persistence of the herbicides chlorsulfuron and metsulfuron-methyl in prairie soils. *Bulletin of Environmental Contamination and Toxicology,* **37**, 698-704.

Walker, A. (1987) Evaluation of a simulation model for prediction of herbicide movement and persistence in soil. *Weed Research,* **27**, 143-152.

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

Walker, A.; Cotterill, S.J.; Welch, S.J. (1989) Adsorption and degradation of chlorsulfuron and metsulfuron-methyl in soils from different depths. *Weed Research,* **29**, 281-287.

THE RELATIVE IMPORTANCE OF ADSORPTION AND DESORPTION CONSTANTS TO THE ASSESSMENT OF PESTICIDE LEACHING POTENTIAL IN SOIL

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ABSTRACT

Mathematical models are being increasingly used to predict the behaviour of pesticides in soils. Models are becoming more sophisticated as scientists widen their knowledge of the fundamental properties that determine environmental fate in soil. This paper draws attention to adsorption-desorption phenomena. These are key processes defining the distribution of pesticides between the dissolved and the sorbed phase in soil and differentiate between the available and unavailable portion of an applied compound determining the movement into deeper soil layers and possible entry into the ground water. Adsorption and desorption coefficients, usually measured in laboratory experiments, provide specific values for a combination of compound and soil. Frequently K_{oc} values are employed in computer models for non-ionic agrochemicals obtained from transformations of the Freundlich equation and normalisation according to the organic carbon content of the soil used. One phenomenon which is frequently observed in laboratory adsorption/desorption studies is hysteresis, originating from incomplete reversibility of the adsorption process. Several types of hysteresis effects are demonstrated for a range of compounds and the possible impact on modelling results are considered. A better understanding of this aspect should result in increased accuracy of modelling of pesticide mobility in soils.

INTRODUCTION

The movement of a pesticide in soil is primarily dependent upon molecular structure causing the compound to exhibit properties due to the presence of certain functional groups. These functional groups govern the partitioning properties of the molecule between the soil particles and the soil solution. Pesticides move primarily from the surface soil layers down the soil profile in solution form and therefore an understanding of the factors that influence retention of the compound by the soil matrix is crucial to modelling the behaviour in soil. The increasing use of models to estimate the distribution of pesticides in the environment, together with the use of fixed trigger values by regulators causes the focus of attention on our understanding of the adsorption and desorption processes.

MECHANISMS OF ADSORPTION AND DESORPTION

The distribution of a pesticide molecule between the soil solution and the solid surface under field conditions is a dynamic process that involves the continual shifting of the equilibrium process. Organic molecules can be sorbed, i.e. retained on or in the soil matrix, by a wide variety of forces including physical/chemical bonding such as Van der Waals forces, H-

bonding, dipole-dipole interactions, ion exchange, cation bridging and water bridging. Initially it is likely that the mechanism retaining the molecule is the one that provides the first contact between the molecule in solution and the sorbate. With time, however, the type of sorption mechanism responsible for retaining the molecule is likely to change and the molecule becomes more tightly incorporated into the soil matrix. The effect of increasing adsorption with time has been recognised by a number of scientists (Gottesbüren et al., 1994; Walker, 1987) but remains largely unquantified. Diffusion rates of molecules in solution and the accessibility of the adsorption sites play a role in determining the rate of concentration decline in solution. The ability of soil micro-organisms to achieve metabolic transformation is also dependent on retention and diffusion rates.

In field soils the influence of macro and micro pores, non-continuity of pores and channels leading to water pockets together with water content changes causing increasing and decreasing concentration of dissolved organic compounds and nutrients combine to create a very dynamic environment that never reaches a stable equilibrium.

LABORATORY DETERMINATION OF ADSORPTION AND DESORPTION

Under laboratory conditions the determination of adsorption and desorption constants is obtained using a slurry of soil and water. International guidelines exist (OECD, 1981) although there are a number of variations on the basic methodology. A range of concentrations are used commencing at around 50 % of the molecule's water solubility and covering several orders of magnitude. With the use of radio-labelled material the sensitivity of the analysis is significantly enhanced and following a series of desorption stages the quantity of material remaining adsorbed to the soil is obtained by a total combustion process. This enables a complete radioactive recovery to be obtained thus validating the measurements made at each intermediate stage. The experiment is also validated by determining the time to reach both adsorption and desorption equilibrium as well as sorption to the test vessel. The stability of the pesticide in the soil/0.01 M $CaCl_2$ slurry is obtained by solvent extraction and radiochromatographic analysis.

The adsorption coefficient is then calculated by plotting the adsorbed concentrations in soil against the solution concentrations. Traditionally the empirical Freundlich equation has been used with the correlation coefficient as an indicator of the "goodness of fit".

Freundlich Equation

$$C_S = K \, C_W \,^{1/n}$$

where C_S is the quantity adsorbed per unit soil weight
C_W is the concentration in solution
K is the adsorption constant
$1/n$ is a constant

The two independent constants K and 1/n depend upon the soil and the compound. For the adsorption line the 1/n value is generally between 0.8 and 1.0 and is only valid for the experimental range of concentrations. The Freundlich equation takes no account of the filling of the adsorption sites as the solution concentration increases, but because of the use of low solution concentrations this is probably not too great an assumption.

Desorption is measured under laboratory conditions by either partial or almost total removal of the supernatant solution and replacement with fresh untreated solution. Following the establishment of a new equilibrium the distribution between soil and solution is obtained. This may be repeated several times and provides information on how readily the compound is removed from the soil.

RESULTS OF ADSORPTION AND DESORPTION EXPERIMENTS

Adsorption experiments thus provide data on the distribution between the solid and solution phase over a range of concentrations. This simple distribution coefficient commonly referred to as $K_d = C_s/C_w$ varies with compound, soil type and concentration.

Thus a K_{ads} constant obtained over a range of concentrations is only valid over the range studied. The K_{ads} may be converted into a second constant K_{oc} using the following equation:

$$K_{OC} = \frac{K_{ads} \times 100}{\% \text{ organic carbon}}$$

The K_{oc} value assumes that all the adsorption is related to organic carbon fraction of the soil. For non-ionic pesticides in medium to high organic carbon content soils (> 1 %) a good correlation is usually obtained. However, for soils with an organic carbon content below 1 % the K_{oc} approach is less accurate. The K_{oc} value has been found to be the most commonly used coefficient in models (Wagenet and Rao, 1990).

HYSTERESIS EFFECTS

The K_{oc} approach makes several theoretical assumptions of which two are most important in the context of hysteresis. Firstly, an equilibrium is reached between sorbed and dissolved pesticide molecules, and secondly, the adsorption process is completely reversible. Both hypotheses presume rather weak binding between the non-ionic chemical and the organic carbon of soil to allow more or less unimpeded exchange between both. However, hysteresis effects seem to indicate that this may not be applicable to all combinations of compounds and soil types. Figure 1 shows the sorption characteristics of three different compounds in soil obtained from adsorption/desorption studies. The data points for several concentration levels of adsorption and of different desorption steps were connected to form isotherms. Compound A shows almost comparable adsorption and desorption isotherms with only minor hysteresis effects. Both compound B and C are more resistant to desorption and reveal significant differences between adsorption and desorption.

FIGURE 1. Adsorption and desorption characteristics of three compounds representing different types of sorption behaviour.
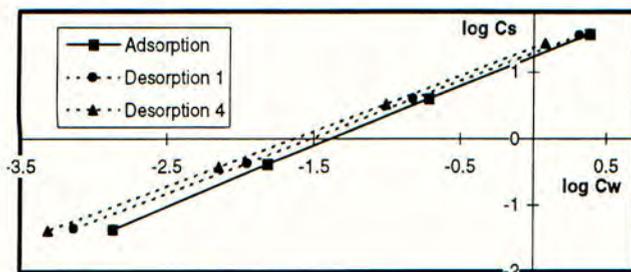
Compound A: Desorption isotherms comparable to adsorption isotherm (minor hysteresis effects).
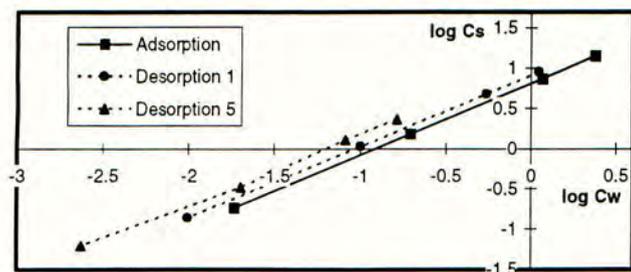
Compound B: Significant hysteresis with notable decline in soil concentrations.
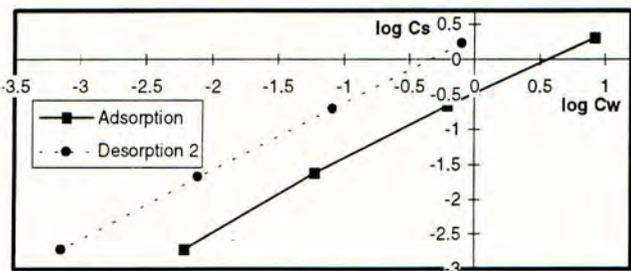
Compound C: Significant hysteresis with almost no decline in soil concentrations.

The mechanisms of hysteresis are not well understood (Koskinen and Harper, 1990). Some researchers have presumed that hysteresis effects are artificial, caused by the laboratory techniques of centrifugation, decanting, etc. or by degradation of the chemical during the experiment. Others found that the non-attainment of equilibrium retarded the desorption process. Wauchope and Myers (1985) applied a sequential-equilibrium model to some compounds assuming that two equilibria exist simultaneously, one for labile and one for restricted soil sites. This means that for a portion of an applied compound, the desorption rate is much smaller than the adsorption rate. Complete irreversibility of sorption represents another situation showing such a strong binding between the compound and the soil that no or very little desorption is possible.

TABLE 1. K_{ads}, K_{des} and K_{oc} values of Compound A, B and C calculated from adsorption and desorption isotherms.

	Compound A	Compound B	Compound C
Adsorption			
K_{ads}	17.15	6.52	0.31
K_{oc}	267	435	82
Desorption 1			
K_{des}	19.19	8.10	-
K_{oc}	301	540	-
Desorption No	4	5	2
K_{des}	22.86	10.76	2.19
K_{oc}	358	718	589

FIGURE 2. Leaching behaviour of Compound B and C in surface layer of the 'Borstel' soil modelled by PELMO using different K_{oc} values obtained from the corresponding adsorption and desorption isotherms.

Compound B (12 month post-application)
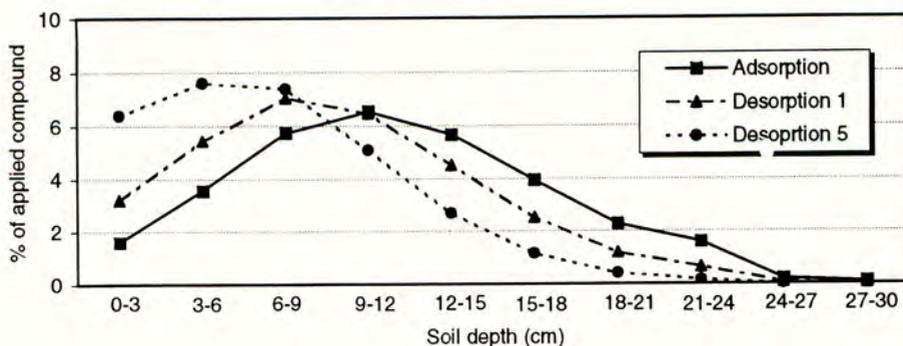
Compound C (4 month post-application)
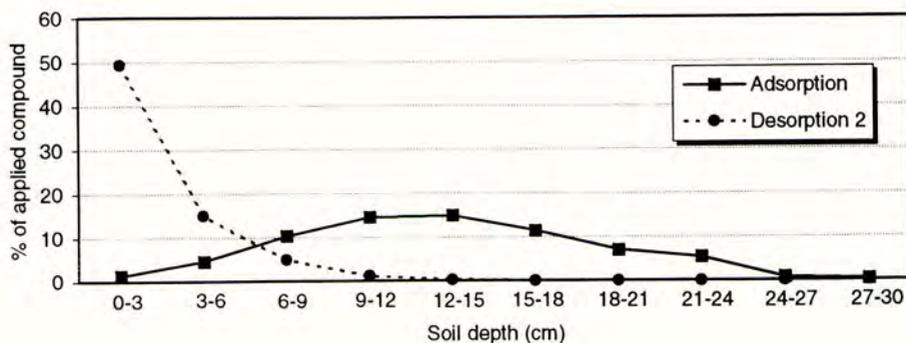
The occurrence of significant hysteresis in adsorption/desorption studies raises the question whether it is necessary to account for this mechanism in modelling the environmental behaviour of agrochemicals in soil. It is apparent from the technical approach of laboratory sorption experiments that hysteresis happens within a relatively short period of time (several hours to a few days). In relation to the field situation where much longer time scales (several weeks to months) need to be considered, the time for significant hysteresis measured in the laboratory seems to be very short. Underestimation of sorption and overprediction of the movement of pesticides in the soil is often observed when compairing field data and model simulations. Therefore, where significant hysteresis occurs, the use of the desorption coefficient in place of the adsorption coefficient may improve the accuracy of modelling.

The K_{oc} values shown in Table 1 were used to simulate the movement of the compounds B and C with the model PELMO Version 1.5 (Klein, 1993) to illustrate the implications of higher sorption as indicated by hysteresis effects (Figure 2). Compound B had a DT_{50} value of 50 days and compound C a DT_{50} value of 150 days. The movement in the 0-30 cm surface soil layer divided into 3 cm increments is shown in Figure 2. Soil (Borstel) and weather scenarios (Grafschaft 1968) were chosen to simulate 'worst case' conditions (German scenarios are implemented into the model). The movement of both compounds in soil is significantly reduced using K_{oc} values calculated from desorption isotherms.

CONCLUSION

Hysteresis is fairly frequently observed in adsorption/desorption studies. The occurence indicates that the adsorption is not fully reversible and that the retention of pesticides by soil may be underestimated. This underestimation of sorption results in overprediction of pesticide movement as shown by model simulations. A better understanding of hysteresis and the kinetics of desorption should result in increased accuracy of modelling science.

REFERENCES

Gottesbüren, B., Pestemer, W., Beulke, S. (1994): Characteristics and effects of the time course of the sorption behaviour of herbicides in soil. Book of Abstracts, Vol. 1, Eighth IUPAC Congress of Pesticide Chemistry. Washington, DC. p. 261.

Klein, M. (1990): PELMO Pesticide Leaching Model. Version 1.5. Schmallenberg.

Koskinen, C. W., Harper, S. S. (1990): The retention process: Mechanisms. In: Pesticides in the Soil Environment: Processes, Impacts, and Modeling. SSSA Book Series 2, H. H. Cheng (ed.), Madison, Wisconsin, 51-77.

OECD (1981): OECD Guidelines for testing of chemicals. Part 106 Adsorption/Desorption. OECD Environment Directorate, Paris.

Wagenet, R. J., Rao, P. S. C. (1990): Modeling pesticide fate in soil. In: Pesticides in the Soil Environment: Processes, Impacts, and Modeling. SSSA Book Series 2, H. H. Cheng (ed.), Madison, Wisconsin, 351-399.

Walker, A. (1987): Evaluation of a simulation model for prediction of herbicide movement and persistence in soil. Weed Res., 27, 143-152.

Wauchope, R. D., Myers, R. S. (1985): Adsorption-desorption kinetics of atrazine and linuron in fresh water-sediment aqueous slurries. J. Environ. Qual., 14, 132-136.

SORPTION AND DESORPTION OF TEFLUTHRIN INSECTICIDE BY SOIL UNDER SIMULATED RAINFALL RUNOFF CONDITIONS

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ABSTRACT

Tefluthrin sorption and subsequent desorption from soil particles were studied in a simulated rainfall runoff system. Sorption results showed that the partition coefficients (K_p, K_{oc}) were determined with good precision with a RSD of 2.8 - 3.0%. Desorption kinetic studies using a centrifugation method indicated a biphasic desorption process in the aqueous phase while the soil phase concentration decreased with time. A direct particle counting method was shown to be able to rapidly measure desorption at varying conditions. Desorption was shown to be rapid and temperature had a pronounced effect on desorption.

INTRODUCTION

Pyrethroid insecticides are active foliar contact insecticides representing 20% of the world insecticides market (Perrior, 1993). The history of progress of development of pyrethroid insecticides has been comprehensively reviewed (Elliott et al., 1978; Perrior, 1993). Tefluthrin, the first soil-active pyrethroid, is especially valuable against corn rootworms and cutworms in the USA and is used to control a range of pests on maize and sugar beet in western Europe.

Pyrethroid insecticides are mainly used as agricultural and horticultural insecticides, however they may still enter aquatic environment via spray drift and soil runoff (Hill, 1989). The fate of pyrethroids in the aquatic environment is regulated by many processes and mechanisms, of which sorption to soils, suspended particles and sediments is probably the most important as shown in our recent studies (Zhou et al., 1994; 1995). Indeed, pyrethroids discharged to bodies of water are present mainly in the adsorbed state where they are substantially less bioavailable and less toxic than in the aqueous phase (Hill, 1989).

In aquatic environments the presence of suspended particles and bottom sediments further reduces the aqueous concentration of pyrethroids and hence their bioaccumulation

potential. However some concern has been expressed that sorption may be partly reversible and desorption may occur under certain environmental conditions. Desorption may lead to increased water concentrations, albeit transient, and pose a potential hazard.

The objectives of this study were: (a) to evaluate reproducible methods suitable for the study of the desorption of pyrethroids from soil, focusing on tefluthrin as a widely used pyrethroid and (b) to use such methods to derive kinetic and thermodynamic parameters for the desorption of tefluthrin from environmentally realistic suspensions of representative soils.

EXPERIMENTAL

Soil preparation and characterisation

A silty clay loam from Champaign, Illinois, USA (0 - 10 cm) was used in this study as it is representative of the soils in Midwest USA which is one of the most extensive agricultural regions in the Country and where tefluthrin has been widely applied for pest control. The soil contained 3.3% organic matter, the CEC was 0.233 meq/g and pH 5.5.

Organic compounds

Carbon-14 labelled tefluthrin was supplied by Zeneca Agrochemicals (Jealott's Hill Research Station). The final purity was determined to be greater than 99% with a specific activity of 2.1 GBq/mmol. The identity of the trace compound in the aqueous and soil phases was checked periodically by radio-TLC (Berthold 2π scanner) on silica gel using hexane/diethyl ether (95/5) as the mobile phase throughout the experiments. As a source of DOC, a well-characterised fulvic acid fraction was obtained from Dr. M. H. B. Hayes, University of Birmingham. This was isolated from the River Dodder, Dublin, Eire using usual XAD resin fractionation method.

Sorption experiments

A mixture of tefluthrin (6 or 0.6 μg), Milli-Q water (30 ml) and soil (2.0 g) prepared in 30 ml glass centrifuge tubes was shaken in the dark for 24 h to reach sorption equilibrium. Three replicates of each treatment were prepared; two of these were used to determine the distribution of tefluthrin between soil, water and glass wall while the other used for desorption experiments. In addition, two blanks containing only soil in water were also analysed to check background radioactivity and final pH in suspension. After equilibrium, two of the centrifuge tubes were removed and centrifuged (RCF 2330 g) for 20 min. Duplicate supernatant samples (4 ml) were mixed with 16 ml of scintillation cocktail and measured by liquid scintillation counting (LSC). The rest of the suspension was then filtered through a 0.45 μm membrane filter. Portion of the solids were weighed and measured by LSC.

Desorption experiments

Centrifugation method

A glass desorption tank containing an appropriate amount of Milli-Q water was contained in a water bath, which was connected to a Churchill thermostat unit. The temperature in the glass desorption tank was kept constant. When the dilution medium had been prepared, the contents of one centrifuge tube were carefully transferred to the desorption tank, the final volume of which was 3.5 l. The solution was stirred constantly. Duplicate samples (25 ml) were taken regularly and centrifuged immediately at 4000 rpm for 10 min and 4 ml aliquots of the supernatant were measured by LSC. In one tube the soil and water were resuspended and discarded, the tube dried and extracted with 3 x 10 ml dichloromethane (DCM). For the second tube, the supernatant was carefully discarded, with the soil and glass wall extracted with 3 x 10 ml DCM. The DCM extracts were late counted by LSC. In doing so, the distribution of tefluthrin in various phases can be quantified.

Direct particle counting method

The desorption tank was set up in exactly the same way as in the centrifugation method. Initial pH was adjusted to different values to study the effect of pH on desorption kinetics; different amounts of 2M NaCl were added to prepare different ionic strength media and different volumes of 1000 mg/l fulvic acid were added to study the effect of DOC on desorption kinetics. Samples were taken regularly and filtered through a 0.45 μm membrane filter. Soil particles plus the filter paper were mixed with 10 ml 'filter-count' and measured by LSC. Simultaneously two samples were taken to determine the aqueous concentration using the centrifugation method.

RESULTS AND DISCUSSION

Tefluthrin sorption to soil particles

The distribution of tefluthrin among various phases in a centrifuge tube was determined as this is a prerequisite for understanding the subsequent desorption process. The results are shown in Table 1, together with the partition coefficients. The partition coefficients were an order of magnitude smaller than those previously reported for tefluthrin sorption (Hamer et al., 1992; Zhou et al., 1994) and this may be explained by the high soil concentration used, the kinetic limitations, the presence of colloids and the different sorptive capacity of different organic matter.

TABLE 1. Distribution of ^{14}C-tefluthrin among three phases in sorption experiments

Total amount (μg)	Aqueous phase (μg/l)	Soil phase (μg/g)	Glass wall (μg)	Partition coefficients	
				K_p	K_{oc}
6.0	6.659±0.240	2.839±0.019	0.118	426±3	22000
6.0	5.584±0.041	2.833±0.060	0.126	507±11	27000
0.6	1.258±0.028	0.270±0.016	0.013	223±13	11000

When the total amount of tefluthrin added to the system was decreased 10-fold, the equilibrium was different as shown in Table 1. It is clear that both glass wall and soil phase tefluthrin concentrations decreased by approximately 10-fold, while that in the aqueous phase deceased by less than 5-fold. The partition coefficients were therefore half of those found in high soil concentration. This is a further evidence of the presence of colloids which transfer solutes into the aqueous phase from the soil phase.

Desorption kinetics

Centrifugation method

Concentrations of tefluthrin in both aqueous and soil phases with time are plotted in Fig. 1. The time course of the aqueous concentration can be divided into two phases, an initial rapid rise which was linear and lasted approximately 14 min followed by a second non-linear phase which continued for the duration of the experiment (25 h). Rapid desorption in the first phase indicated a fast release of weakly sorbed tefluthrin from soil surfaces as a result of dilution, consistent with the suggestion that this is a reflection of desorption from the fast-equilibrating sorption sites (Kookana *et al.*, 1992; Connaughton *et al.*, 1993). The second phase was a long slow process in which the aqueous concentration decreased gradually due to serious tefluthrin sorption to the glass walls. This is different from the second phase normally found for other compounds which shows a slow increase in aqueous concentration due to desorption from the strong binding sites. It is quite possible that such sites are present on the Champaign soil, however, a clear picture of tefluthrin release from such sites was masked by the sidewall effect.

Fig. 1. Tefluthrin desorption study using the centrifugation method. (○) aqueous phase, (■) soil phase, (▼) glass wall.
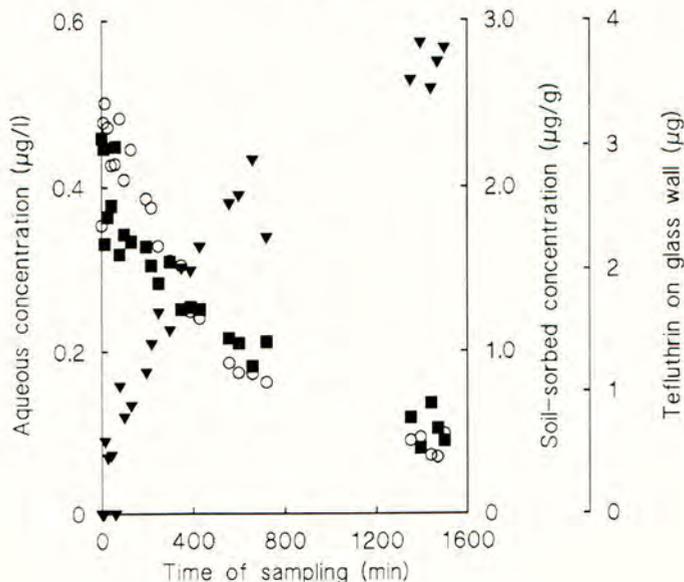
Soil-sorbed tefluthrin concentration showed a singular trend of decrease in quantity from 2.3 µg/g at time 0 to 0.4 µg/g at time 25 h, a decrease of 80%. The results therefore confirmed that a desorption process was taking place continuously throughout the experimental period.

If the changes in aqueous and soil phase tefluthrin concentrations are examined together, it becomes clear that tefluthrin desorbed from soil particles was re-adsorebd onto the glass walls. To confirm this hypothesis, the amount of tefluthrin sorbed on glass walls was calculated at each sampling point and the results are shown in Fig. 1. The results show clearly increased sorption to glass walls with time, approaching 63% of the total tefluthrin added.

Direct particle counting method

Desorption kinetic results at pH 5.23, 5.86 and 6.45 are shown in Fig. 2. It is clear that solution pH did not have a significant impact on desorption kinetics or equilibrium soil-sorbed tefluthrin concentration. Statistic analysis of the desorption curves at different pH's using 95% confidence intervals showed that desorption at these three pH's was not significantly different. As a result, the effect of pH is trivial. However, more work needs to be done over a wider pH range before a conclusion can be achieved. Similarly the effects of ionic strength and DOC on desorption kinetics were also found to be relatively small.

Fig. 2. Desorption kinetic study using the direct particle counting method. (■) pH = 5.23, (○) pH = 5.86, (△) pH = 6.45.
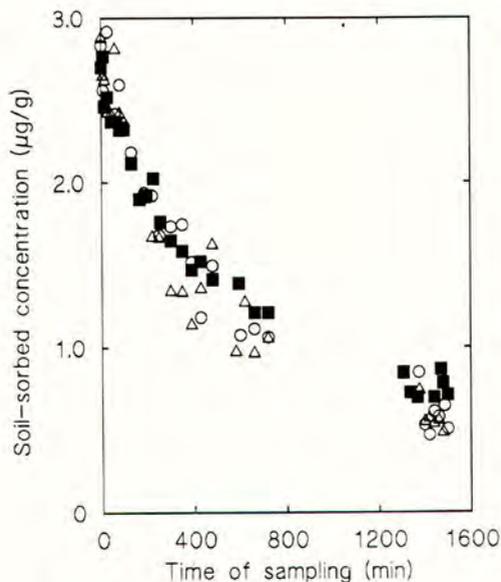
<u>Effect of temperature on desorption</u>

Desorption experiments were repeated at three different temperatures, from which thermodynamic data were obtained. It is shown in Fig. 3 that the rate of desorption increased with increase in temperature, indicated strongly by the first order modelling curves:

$$q = \frac{3.7953 \, k_a / M}{k_d + k_a - 0.001673} \, e^{-0.001673 \, t} + K \, e^{-(kd + ka) \, t} + q_e$$

where q is soil-sorbed tefluthrin concentration (μg/g); q_e is equilibrium soil-sorbed concentration (μg/g); k_d and k_a are desorption and sorption rate constants (min^{-1}), respectively; M is the mass of soil (g); t is time (min).

Results in Table 2 showed that both the equilibrium soil phase concentration and the partition coefficient (K_p) decreased with increase in temperature while the desorption rate constant increased with temperature. The results are therefore consistent with many reports that decreasing temperature favours sorption of certain hydrophobic organic chemicals and point to a physical sorption mechanism (Szecsody and Bales, 1991).

Fig. 3. Desorption kinetic study using the direct particle counting method. (o) 20°C, (■) 15°C, (▽) 9°C.
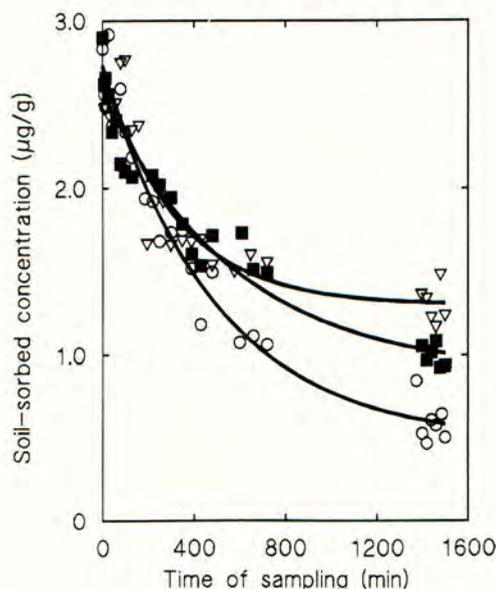
TABLE 2. Desorption kinetics at three different temperatures

Temperature (°C)	q_e (μg/g)	K_p	k_a (min^{-1})	k_d (min^{-1})
9	1.153	13894	0.00229	0.00029
15	0.863	8267	0.00257	0.00055
20	0.419	7072	0.00358	0.00089

Thermodynamic parameters were calculated using the following equations:

$$\Delta G = - R \, T \ln K_p$$

$$\ln K_p = \frac{\Delta S}{R} - \frac{\Delta H}{RT}$$

The negative value of enthalpy confirms the exothermic nature of tefluthrin sorption to soil particles and is consistent with the partition mechanism (Chiou et al., 1979). The negative values of free energy indicate that tefluthrin sorption is spontaneous. The negative values of entropy suggests the decreased randomness at the soil - solution interface during sorption of tefluthrin on soil particles. In the process of sorption, solute molecules lost entropy during transition from the solution phase to solid surfaces and such a loss was greater than the translational entropy gained by adsorbed solvent molecules.

CONCLUSIONS

Sorption experiments showed about 95% of the tefluthrin was sorbed onto the soil particles and only a small percentage (< 3%) was left both in the water and on the tube walls. These results are as expected and in agreement with those which we reported previously for the sorption of tefluthrin and lambdacyhalothrin to organically-coated mineral particles rather than soil (Zhou et al., 1994, 1995).

In the desorption experiments, the centrifugation method yielded information for both the aqueous and soil phase concentrations, which indicated a fast desorption process accompanied by a strong glass wall sorption process. The amount and kinetics of wall sorption calculated via mass balance showed it to be exponential in form.

Combining the direct particle counting method and the centrifugation method enabled fast measurement of both aqueous and soil phase tefluthrin concentrations. Soil phase concentrations were found to decrease with time, approaching equilibrium at 24 h. The desorption process was well modelled by reversible first order kinetics, and was amenable to theoretical derivation based on kinetic, equilibrium and mass balance calculation.

The variables examined included pH, ionic strength, DOC and temperature, which are all important in aquatic and soil environments. It has been shown that desorption increased with temperature while the effects of pH, ionic strength and DOC were not significant. Further work is needed to study desorption in the presence of suspended particles; to examine the effects of soil composition and ageing on desorption kinetics; to carry out desorption experiments in real runoff events by modifying the present simulation scheme.

ACKNOWLEDGEMENT

REFERENCES

Chiou, C.T.; Peters, L.J.; Freed, V.H. (1979) A physical concept of soil-water equilibria for nonionic organic compounds. *Science (Washington, D.C.)*, **206**, 831-832.

Connaughton, D.F.; Stedinger, J.R.; Lion, L.W.; Shuler, M.L. (1993) Description of time-varying desorption kinetics: release of naphthalene from contaminated soils. *Environmental Science and Technology*, **27**, 2397-2403.

Elliott, M.; Janes, N.F.; Potter, C. (1978) The future of pyrethroids in insect control. *Annual Review of Entomology*, **22**, 443-469.

Hamer, M.J.; Maund, S.J.; Hill, I.R. (1992) Laboratory methods for evaluating the impact of pesticides on water/sediment organisms. *Brighton Crop Protection Conference - Pests and Diseases 1992*, 487-496.

Hill, I.R. (1989) Aquatic organisms and pyrethroids. *Pesticide Science*, **27**, 429-465.

Kookana, R.S.; Aylmore, L.A.G.; Gerritse, R.G. (1992) Time-dependent sorption of pesticides during transport in soils. *Soil Science*, **154**, 214-225.

Perrior, T.R. (1993) Chemical Insecticides for the 21st century. *Chemistry & Industry*, **22**, 883-887.

Szecsody, J.E.; Bales, R.C. (1991) Temperature effects on chlorinated-benzene sorption to hydrophobic surfaces. *Chemosphere*, **22**, 1141-1151.

Zhou, J.L.; Rowland, S.J.; Braven, J.; Mantoura, R.F.C.; Harland, B.J. (1994) Tefluthrin sorption to mineral particles: role of particle organic coatings. *International Journal of Environmental Analytical Chemistry*, in press.

Zhou, J.L.; Rowland, S.J.; Mantoura, R.F.C. (1995) Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. *Water Research*, in press.

THE USE OF MINI-LYSIMETERS TO STUDY THE INFLUENCE OF RAINFALL INTENSITY ON PESTICIDE TRANSPORT AND WATER PATHWAYS

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ABSTRACT

An experiment was set up to study the influence of rainfall intensity on pesticide transport and vertical by-pass flow in a heavy clay soil of the Denchworth series. The Mini-lysimeters were 20 cm diameter by 20 cm deep and had the edges sealed with molten vaseline. Both isoproturon and the bromide tracer were applied to the surface of the mini-lysimeters prior to irrigation in the laboratory. Two irrigation rates were used, which were equivalent to 2.6 and 10.5 mm/h rainfall to deliver the same total volume to the columns. As expected leachate was collected from the column receiving the high intensity irrigation treatment. The appearance of pesticide and tracer in the very first fractions collected illustrated the importance of by-pass flow in this soil. The pattern of release of pesticide from the soil differed between the two irrigation rates. The high intensity treatment generated a peak concentration which then fell with each subsequent fraction, whilst with the low intensity treatment the concentrations of pesticide in the leachate was almost constant. The fourfold difference in irrigation intensity resulted in a tenfold difference in losses of pesticide and bromide tracer. Application of rhodamine B dye to one of the columns revealed a wide variety of macropores capable of transporting water in the top 4 cm of the soil but that from 13 cm downwards worm burrows were dominant.

INTRODUCTION

Many field experiments have shown that the initiation of preferential or by-pass flow in certain soils (eg. heavy clay) will to lead to the transport and loss of significant amounts of pesticide to surrounding water courses (Johnson *et al.*, 1994). However, for a given rainfall event on a soil in which the total amount of pesticide present is known, there are numerous factors which govern the amount of pesticide lost, such as antecedent soil conditions, preceding rainfall events and rainfall volume. An important factor will be rainfall intensity. Rainfall intensity is believed to influence the speed of breakthrough (Edwards *et al.*, 1992) and to cause more macropores to convey water (Edwards *et al.*, 1993). However, it was suggested that rainfall intensity would not influence pesticide concentration (Edwards *et al.*, 1992; Edwards *et al.*, 1993). It is difficult to extend the work done by Edwards and his colleagues to the UK as the rainfall intensities used were very high, the lowest being equivalent to 15 mm/h.

In the field it is difficult to ascertain the importance of these different factors on the amount of pesticide lost simply because we cannot manipulate the weather conditions (without a great deal of expense). Whilst lysimeters containing undisturbed soil have their limitations, they offer are an excellent tool with which to study some of these questions. At the fieldsite

at Wytham, Oxfordshire (heavy clay soil), from which the mini-lysimeters were taken, the soil hydrology has been studied for two seasons. This research has shown (Haria *et al.*, 1994) that drain flow, from where the majority of pesticide losses occurred, is linked to the development of saturation and a perched water table in the topsoil. Against this background it is not unreasonable to use a free draining lysimeter in a saturated flow situation to simulate these conditions. In this experiment mini-lysimeters were used to determine the influence on the release of solutes from the soil surface by rain falling at different intensities.

MATERIALS AND METHODS

Mini-lysimeters were specially constructed to a design similar to that described by Cameron et al. (1990). They consisted of 20 cm by 20 cm diameter aluminium tube with bevelled cutting edge and an inner collar at the cutting edge of 3 cm in height, which reduced the inner diameter. The technique used to collect these mini-lysimeters involved digging near the plot, and exposing a soil island over which the aluminium container was slid. The effect of the inner collar was to leave a 1 cm gap all round between the soil and the tube which was then filled with molten vaseline. Once the vaseline had solidified the mini-lysimeters were removed and attached to the base containing a stainless steel mesh. These columns were taken from the field near the plot in December 1993 and January 1994 when the topsoil was close to saturation.

Prior to starting the experiment the columns were brought close to saturation by adding small amounts of 2 mM calcium chloride. Before the experimental irrigation began a small soil sample was taken from the surface of one of the columns to determine the soil moisture content. The replicate mini-lysimeters were exposed to 2 different rainfall intensities, but the same rainfall volume and antecedent conditions (close to saturation). Isoproturon (in a commercial formulation) was applied at twice normal field rate together with bromide tracer. Irrigation commenced 15 min after application of the pesticide and tracer, at rates equivalent to 10.5 and 2.6 mm/h. The Irrigation treatments involved two peristaltic pumps to deliver the eluent of 2 mM Calcium chloride to the two different sets of mini-lysimeters. The 'rainfall' was was applied via 4 irrigation tubes (position of each nozzle changed every 10 min) which were held 15 cm above the soil surface. 6 x 65 ml samples were collected on first day. Repeated the following day, a total of 13 samples were collected. The low irrigation rate experiment was repeated with a third column (Lc) using irrigating tubes held in a rotary shaker, thus further randomising the spread of water droplets, otherwise conditions the same as before for the low irrigation treatment. The leachate samples were analysed for isoproturon and anions by HPLC. Aliquots of soil (50 g) from columns La and Hb were extracted with calcium chloride (anions) or methanol (isoproturon) prior to analysis. 0.5% Rhodamine B dye was applied to one of the mini-lysimeters at 8 mm/h via one nozzle until 2000 ml leached through the core before it was sectioned and photographed. The amount of soil stained with depth was assessed by eye using a grid and tracing paper.

RESULTS AND DISCUSSION

The experiment with mini-lysimeters was set up with the aim of assessing the influence of rainfall intensity on pesticide transport (although it was not possible to achieve the same kinetic energy of natural rainfall impacts). Over the 2 days of the experiment about 1060

ml was applied to each column (0.45 of pore volume), with 845 ml being eluted from each. As can be seen in Table 1 leachate was collected from the high intensity rainfall treatment (40 min) before the low intensity (110 min). The appearance of pesticide and tracer in the first fractions collected illustrated the dominance of by-pass flow. As the leachate only represented 0.45 of a pore volume the arrival of the solutes in the leachate is not related to a 'true breakthrough curve'.

TABLE 1. Leachate collection information (mean of 2 replicate columns, all data in minutes)

	Delay before draining starts	65 ml collection interval	Time required to collect 6 fractions
10.5 mm/h day 1	40	12	112
10.5 mm/h day 2	19	12	91
2.6 mm/h day 1	110	54	434
2.6 mm/h day 2	65	52	377

The soil columns which received the high intensity irrigation treatment yielded 6 % of the applied isoproturon, whilst the columns which received the low intensity irrigation treatment yielded 0.5 % of the applied dose (Table 2.).

TABLE 2. Loss of isoproturon (ipu) and bromide (Br) from the mini-lysimeters as a percentage of the original applications. The results are the means of two replicates with the exception of the 2.6 mm/h ro which represents a single mini-lysimeter in which the rainfall was applied with the aid of a rotary shaker.

	2.6 mm/h irrigation				10.5 mm/h irrigation	
	ipu	ipu ro	Br	Br ro	ipu	Br
Day 1	0.19	0.05	1.10	12.00	3.75	16.10
Day 2	0.28	0.26	1.75	21.00	2.10	12.60
Day 3	ND*	1.24	ND	16.60	ND	ND
Total	0.46	1.55	2.80	49.00	5.85	28.60

ND* Irrigation not carried over for a third day with these treatments.

An additional calculation for isoproturon can be made using the Kd of 2.5 which has been measured for isoproturon in Wytham soil. Assuming an equilibrium exists between the aqueous and sorbed phases of isoproturon then,

$$S = kd*C \tag{1}$$

where, S is the sorbed isoproturon concentration (μg/kg dry weight), and C is the aqueous

concentration (μg/l). The total pesticide present in any given depth of soil, T, is the total of the aqueous and sorbed phases,

$$T = SM + CV_w \tag{2}$$

In which M is the mass of the soil, V_w is the volume of water, giving,

$$C = T / V_s*(kd * \varrho + \Theta) \tag{3}$$

where, V_s is the volume of soil, ϱ is the bulk density of the soil (kg/l) and Θ (cm/cm) is the soil water content. Thus, if the total pesticide content of a soil volume is known together with its bulk density water content and pesticide soil/water partition coefficient, the aqueous available pesticide concentration and amount can be calculated.

For the purposes of this calculation the applied pesticide (16.37 mg) was assumed to be confined to the top 2 cm of the soil in the column with which it was in equilibrium. Since all of the columns started from the same initial water content and received the same isoproturon dose, then the aqueous available isoproturon was the same in the four columns, 1.78 mg at a concentration of 7.1 mg/l. During the experiment 845 ml of water passed through the column which amounts to several pore volumes of the top 2 cm of soil. The proportion of the aqueous available isoproturon lost from the replicate high intensity rainfall events was 43% and 64%. For the low intensity rainfall events the corresponding proportions were both 4%. Clearly there is a significant difference between the low and high intensity rainfall events.

Thus, a fourfold difference in irrigation rate generated a tenfold difference in isoproturon and tracer for the same volume of water applied. The similarities in the ratios for the isoproturon and bromide losses under the different irrigation intensities suggested the primary factor was physical. In both cases 5 times more bromide was lost than isoproturon illustrating the influence of adsorption of the organic molecule. The release of pesticide appeared to follow different patterns under the different rainfall regimes, with high intensity rainfall yielding an immediate peak followed by decline and low intensity generating an almost steady release of pesticide (see Fig. 1). The loss of both isoproturon and bromide was less on the second day of irrigation than the first, with the high intensity treatment. This phenomenon was also noted by Shipitalo et al. (1990) with atrazine in irrigated soil columns. In contrast the losses of solutes were the same or greater for each succeeding day of irrigation with the low intensity treatment (Table 2).

When considering the mobilisation of solutes from the soil surface (in a clay soil) due to rainfall or irrigation it is worthwhile examining what happens when a drop of water hits the soil. Ahuja and Lehman (1983) suggest that the mechanism of release of pesticide from soil aggregates to the newly arrived rainwater is by turbulent diffusion. This will occur in the area of soil covered by the recently arrived rain drop (perhaps 5 mm in diameter), for a few moments saturated conditions are created within this small area. We can assume that in this small area all the solutes in the aqueous phase are going to be mixed with the new water. If this new water then locates a macropore which can conduct water then the solutes will be relocated perhaps arriving in the drains. This is true only for the solutes in the area of soil hit by the water drop. With a high intensity irrigation regime, the soil infiltration capacity is soon exceeded. A very thin layer of ponded water forms on the entire soil surface thus making all the solutes on the soil surface potentially available for transport.

To increase the realism of the experiment for the low intensity irrigation treatment, the nozzles were rotated by being held in a rotary shaker to generate a more random spread of
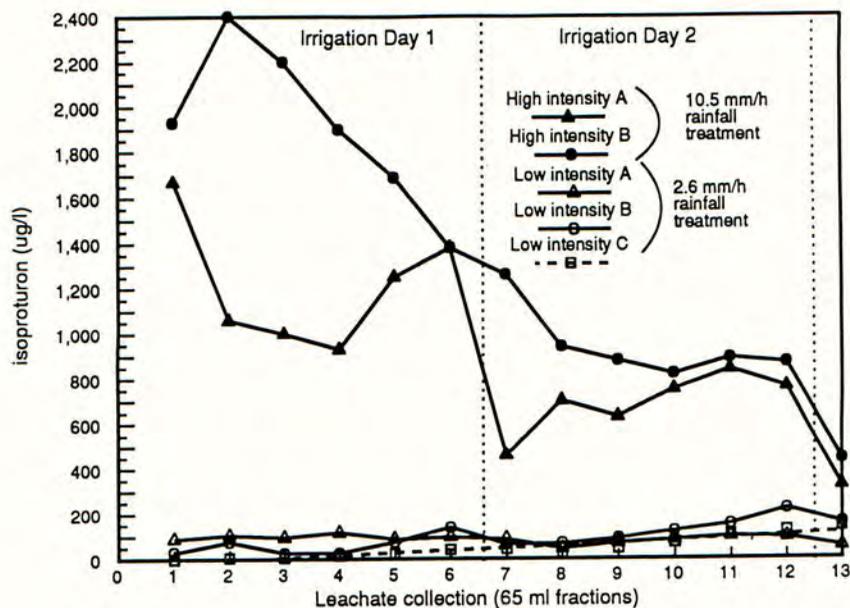
FIGURE 1. Comparison of isoproturon concentrations leached from the mini-lysimeters in response to the different irrigation treatments. Note the time interval between fraction collections was 12 minutes for the high intensity and 52-54 minutes for the low intensity irrigation.
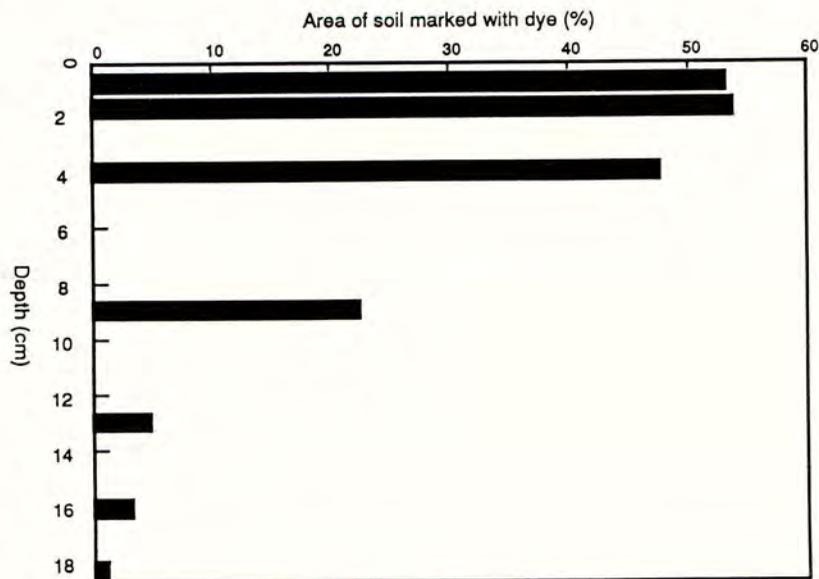
FIGURE 2. Area of soil within a mini-lysimeter marked by rhodamine B dye

water drops. As observed previously the low intensity irrigation treatment was less efficient at transporting pesticide from the soil surface than the high intensity (see Fig. 1). However, in contrast to the previous low intensity irrigation experiment, a much greater proportion of bromide was released (Table 2).

An assessment of the residues left in the soil gave disappointingly low recoveries, which may be the result of degradation prior to analysis and/or poor technique. The results of the dye experiment are shown in Figure 2. Examination of the dye location in the column confirmed that the dye had moved through the column rather than down the edge. In the top 4 cm, which contained an extensive pattern of fractures, up to 50 % of the soil was stained. The macropore network in the upper 4 cm stained by the dye was dominated by small fractures, with barley root hairs and worm burrows being less important. From 9-13 cm only a few worm burrows and fractures were stained. From 16 cm downwards staining was associated with 2-3 worm burrows of 2 mm diameter. Therefore, around 1 % of the area of the soil at the base of the column was associated with the transport of the water. These results show how little of the soil A horizon below 9 cm was associated with macropore flow, and was in a position to interact with transported pesticide.

In conclusion, under similar antecedent conditions, of the two treatments chosen a low rainfall intensity is less efficient in removing and transporting solutes from the soil surface.

ACKNOWLEDGEMENTS

REFERENCES

Ahuja, L.R.; Lehman. O.R. (1983) The extent and nature of rainfall-soil interaction in the release of soluble chemicals to runoff. *Journal of Environmental Quality*, 12, 34-40.

Cameron, K.C.; Harrison, D.F.; Smith, N.P.; McLay, C.D.A. (1990) A method to prevent edge-flow in undisturbed soil cores and lysimeters. *Australian Journal of Soil Research*, 28, 879-886.

Edwards, W.M.; Shipitalo, M.J.; Dick, W.A.; Owens, L.B. (1992) Rainfall intensity affects transport of water and chemicals through macropores in no-till soil. *Soil Science Society of America Journal*, 56, 52-58.

Edwards, W.M.; Shipitalo, M.J.; Owens, L.B.; Dick, W.A. (1993) Factors affecting preferential flow of water and atrazine through earthworm burrows under continuous no-till corn. *Journal of Environmental Quality*, 22, 453-457.

Haria, A.H.; Johnson, A.C.; Bell, J.P.; Batchelor, C.H. (1994) Water movement and isoproturon behaviour in a drained heavy clay soil: 1. Preferential flow processes. *Journal of Hydrology*, 163, 203-216.

Johnson, A.C.; Haria, A.H.; Bhardwaj, C.L.; Völkner, C.; Batchelor, C.H.; Walker A. (1994) Water movement and isoproturon behaviour in a drained heavy clay soil: 2. Persistence and transport. *Journal of Hydrology*, 163, 217-231.

Shipitalo, M.J.; Edwards, W.M.; Dick, W.A.; Owens, L.B. (1990) Initial storm effects on macropore transport of surface applied chemicals in no-till soil. *Soil Science Society of America Journal*, 54, 1530-1536.

APPLICATION OF A NOVEL HIGH-RESOLUTION TOMOGRAPHIC METHOD FOR NON-DESTRUCTIVE CHARACTERISATION OF PORE STRUCTURE IN SOIL CORES

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ABSTRACT

A novel method of three-dimensional X-ray computed tomography has been improved for applications in soil science. This special technique is called "X-ray microtomography". It is a non-medical apparatus which was originally developed as an accessory for electron microscopes in materials science.

In contrast to other tomographic methods used in soil science, soil samples are not scanned in many layers but projected totally in one step. Many such projections are necessary to reconstruct the pore structure in the soil sample. The convolution back-projection is used as the mathematical reconstruction algorithm. Two-dimensional slices can be generated in any direction from the reconstructed three-dimensional data .

The specific advantage in applying this new methodology in soil research is the high resolution achieved by using a microfocus X-ray source in combination with a special CCD camera (CCD = Charge Coupled Device) as an image detector and the special reconstruction algorithm. The X-ray tube has a focal spot of 10 µm. This leads to a very high resolution depending on the geometry. The cone beam geometry used reduces acquisition time and increases the resolution as well. The achievable spatial resolution is about 100 Lp/mm (Lp = Line pairs) for the cubic soil sample used, which has an edge length of 2 cm.

INTRODUCTION

In order to understand pesticide movement from soil to water not only are the physical and chemical properties of the pesticide important but also the physical and structural properties of the soil. More detailed information on the soil structure is necessary especially for estimating ground water contamination on a wide spatial and temporal scale and for interpreting lysimeter studies.

Soil structure in the vadose zone is neither horizontally nor vertically homogeneous. The pore structure especially has a heterogeneous three-dimensional character. Therefore the transport of water and solution through the unsaturated soil cannot be completely described

by a simple chromatographic process (Beven & Germann, 1982). This is demonstrated by results from lysimeter studies as well. In such studies preferential flow is a frequently observed phenomenon. The kinetics of this rapid mass flow is not of a chromatographic nature. Normal one-dimensional simulation models like WAVE (Vancloster *et al.*, 1993), which are based on solving Richards' equation as a generalised Darcy equation by using the soil hydraulic functions, have difficulties in describing situations with such water flow conditions (Gottesbüren *et al.*, 1993). It is not possible to exactly predict potential ground water contamination using these models, which thus have to be improved. Gerke and van Genuchten (1993) suggested an empirical dual-porosity model to simulate the preferential movement of water and solutes in structured porous media (van Genuchten & Wagenet, 1989; Gerke & van Genuchten, 1993). An analogous idea has been realised in the MACRO model (Jarvis, in press). However, the real mechanism of preferential flow is disregarded in these theoretical conceptions because it remains unknown.

In the literature, preferential flow is discussed as an effect of macropores or transport through the soil via fingers. However, the exact processes are not yet understood. It is therefore necessary to experimentally enable a more detailed description of preferential flow and to identify the pathways for the soil solution. In a second step easily obtainable parameters for simulating a transport mechanism of this kind must be discovered. To analyse flow dynamics through soil it is useful to have a non-destructive method of characterising the three-dimensional pore structure in soil cores. Only if this were realised could flow experiments in undisturbed soil cores be interpreted by their pore structure. Therefore experiments were conducted in 1994 to test different tomographic methods for their applicability in soil science. X-ray microtomography is one of those techniques.

THE TOMOGRAPHIC METHOD AND PHYSICAL CONSIDERATIONS

Tomographic scanners in soil science

Many different tomographic methods using X-rays or γ-rays have been applied in soil science up to now. These computerised tomographs are mostly used for non-destructive visualisation of macropores (Warner *et al.*, 1989; Peyton *et al.*, 1992) or to analyse soil bulk density and volumetric water content (Crestana *et al.*, 1986). Dynamic measurements of water distribution in outflow experiments (Hopmanns *et al.*, 1992) or earthworm activity are possible as well. The necessary scanning time is a limiting factor.

The common feature among the methodologies described in the literature is the scanning of soil samples layer by layer. Only some medical devices are able to scan a few layers simultaneously (Rogasik *et al.*, 1994). Nevertheless, there is always an unscanned region between two layers. The soil cores are not completely measured without gaps. Therefore the continuity of macropores cannot be exactly reproduced using these techniques.

Principle of X-ray microtomography

As a non-destructive imaging procedure, X-ray microtomography is structured experimentally into two major phases (Fuhrmann, 1993). First of all the soil sample is penetrated by the electromagnetic radiation and the soil is characterised by the radiation absorption. After this, many such measured two-dimensional projections are used in a

mathematical algorithm to reconstruct the bulk density distribution of the sample. The three-dimensional pore structure becomes visible without any gaps and can be analysed.

Technical design

Figure 1 shows the technical design of the developed microtomography apparatus. A microfocus X-ray system (KEVEX) is used with two selectable focal spots. One spot diameter amounts to 10 µm and the other to 45 µm. The target voltage can be adjusted up to 100 kV and the electron beam current up to 0.5 mA. Therefore this X-ray tube creates a high quanta flux and leads to a very sharp projection of the measured solid on the specific CCD camera. The CCD camera (PRINCETON INSTRUMENTS) is divided into 512 x 512 pixels. Each pixel has an edge length of 20 µm. A water-cooled Peltier effect thermoelectric cooler reduces the temperature within the CCD camera to -50°C for noise reduction. Many projections are necessary. to reconstruct the three-dimensional soil structure. Therefore the soil sample is placed on a rotational stage and projections are taken under different rotation angles. A personal computer (PC) controls the whole measuring procedure. After measurement the data sets are copied on a DEC station 5000/240 with 128 MB main memory for reconstruction.

Figure 1. Technical design of the X-ray microtomography
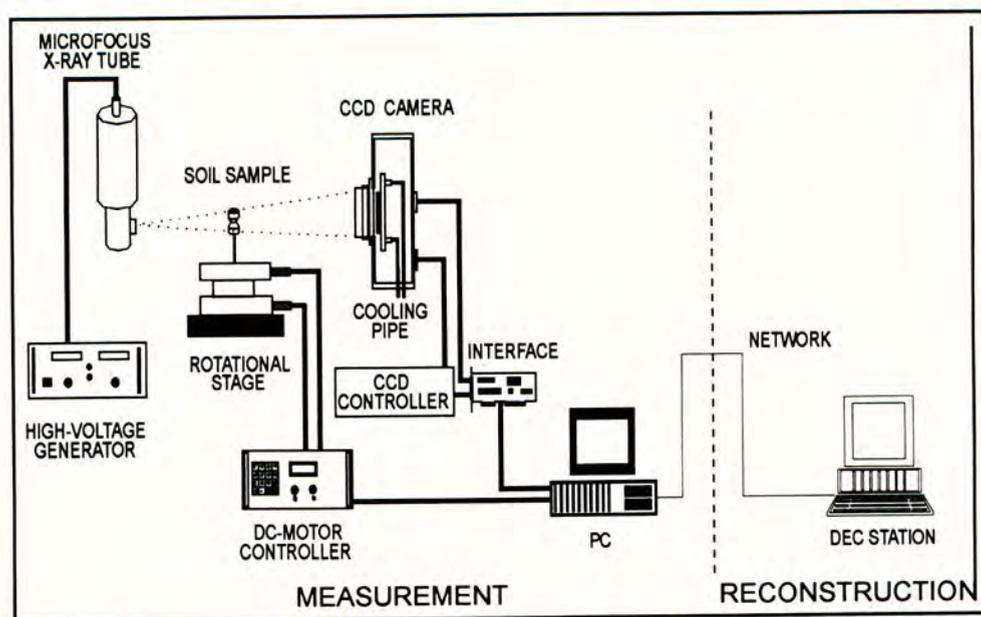
The reconstruction algorithm

In X-ray microtomography a cone beam geometry is utilised to obtain the projections. For the reconstruction of the example tomographies, a specific convolution back-projection algorithm based on Feldkamp was used (Feldkamp *et al.*, 1984; Fuhrmann, 1993). First each raw image of the specimen is subjected to background correction and intensity normalisation

to produce a two-dimensional array of transmission values. Then the projection image is subjected to a geometrical weighting and filtered along horizontal lines with a chosen convolution kernel. The back-projection operation assigns a contribution from each angle of data to each reconstruction point. Finally the contributions from all the angles are weighted and added together. Before the reconstruction can begin the correct axis of rotation must be estimated empirically. A wrongly estimated axis is one reason for artefacts in the reconstructed three-dimensional data.

APPLICATION TO SOIL SCIENCE

Visualisation of pore structure in soil cores

Figure 2 shows a tomographic visualisation of a cubic soil sample, which has an edge length of 2 cm. The soil sample was taken from the lower part of the A_p horizon of an orthic luvisol from Merzenhausen close by Jülich, Germany. The visualisation was made with the public domain software "XDS". Four two-dimensional slices in vertical direction are depicted on the left side. Dark regions have lower radiation absorption then the brighter ones. Therefore soil pores filled with air or water becomes visible as dark regions and little stones as white spots. On the right, the result of isosurface rendering algorithm is depicted showing only the hole rooms in the probe. Only the top third of the soil could be generated as an isosurface because of a shortcoming of the software used. The large hole visible at the margin of the slices and the isosurface is a part of an earthworm burrow. The smallest soil pore which can be identified in this sample must have a diameter of at least 10 µm. This corresponds to a spatial resolution of about 100 Lp/mm (Lp = Line pairs). In order to optimise the resolution the target voltage was set to 90 kV. That makes a medium beam energy of about 45 keV. The electron beam current was set to 0.112 mA. In total 72 projections were made in steps of 5° under these conditions and with a measurement time of 50 s for each projection.

Figure 2. Tomographic visualisation of soil structure. (left four slices, right one isosurface)
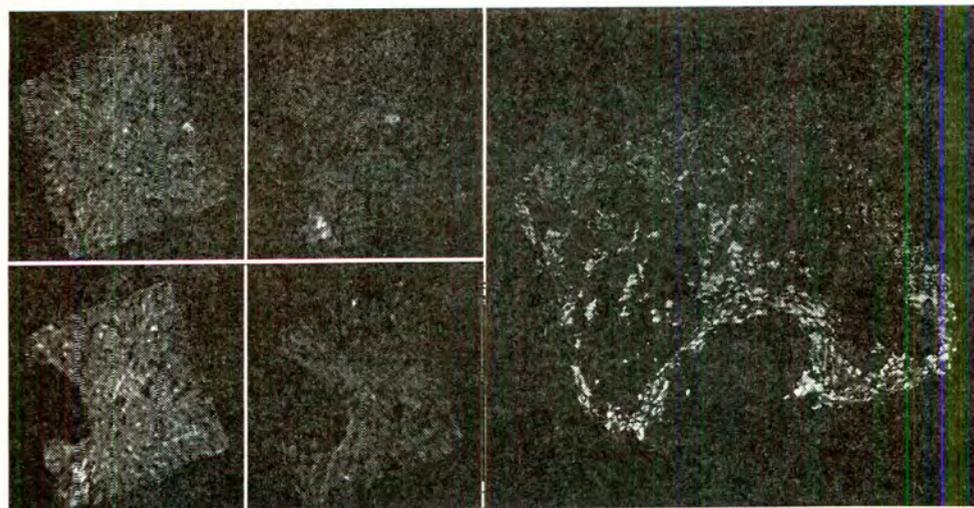
Analysis of macropores in a soil column

Soil columns with a diameter of 8 cm and a height of 6 cm were also measured. These columns are used regularly in outflow experiments (van Dam *et al.*, 1990). In order to measure this bigger sample a different X-ray tube was used with higher target voltage and bigger focal spot. A different scale of enlargement had to be selected for the tomographic projections by changing the distances between the X-ray tube, soil sample and camera. The bigger focal spot and the reduced possibility for geometrical magnification decreases the achievable spatial resolution to 20 Lp/mm. Soil pores can be identified down to diameters of about 50 µm. Figure 3 shows a two-dimensional slice of the reconstructed pore structure (a). Dark regions have lower radiation absorption then the brighter ones. The macropores can be extracted by using digital filter functions which manipulate the image contrast (b).

Figure 3. a) Two-dimensional tomographic slice of the reconstructed pore structure in a soil column with a diameter of 8 cm. b) The same slice after digital image processing.
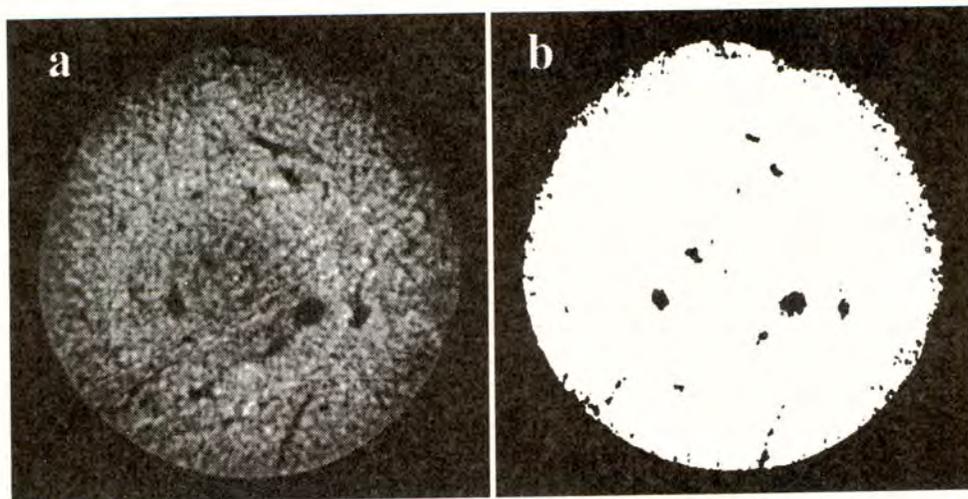
CONCLUSIONS AND PREVIEW

X-ray microtomography is a high-resolution non-destructive imaging technology for application in soil science. It is complicated to use but the soil structure of cores with a scale length from 1 mm to 100 mm can be characterized very well. The continuity and course of the macropores in soil columns, in particular, can be visualised and analysed. The hydraulic functions of tomographically characterized columns can be additionally determined by outflow experiments (van Dam *et al.*, 1990). These measurements lead to better results than estimations from soil properties (Vereecken *et al.*, 1990). A dye penetration experiment in field plots or lysimeters can be performed to characterise regions with preferential pathways through the soil. Soil columns with different unsaturated hydraulic conductivities can be taken. The question is whether the different conductivities correspond to different macropore structures or not. An answer to this question would be a fundamental contribution to analysing the mechanisms of the preferential flow and will be presented soon.

ACKNOWLEDGEMENTS

REFERENCES

Beven, K.; Germann, P. (1982) Macropores and water flow in soils. *Water Resources Research*, **18** (5), 1311-1325.

Crestana, S.; Cesareo, R.; Mascarenhas (1986) Using computed tomography miniscanner in soil science. *Soil Science*, **142** (1), 326-332.

Feldkamp, L.A.; Davis, L.C.; Kress, J.W. (1984) Practical cone-beam algorithm. *Journal of the Optical Soiety of America A*, **1** (6), 612-619.

Fuhrmann, G. (1993) Entwicklung eines Mikrotomographiesystems als Zusatz für Rasterelektronenmikroskope. PhD Thesis RWTH Aachen, Germany. In: *Berichte des Forschungszentrums Jülich*, **2718**.

Gerke, H.H.; van Genuchten, M.T. (1993) A dual-porosity model for simulating the preferential movement of water and solutes in structured porous media. *Water Resources Research*, **29** (2), 305-319.

Gottesbüren, B.; Printz, H.; Pütz, T.; Mittelstaedt, W.; Führ, F. (1993) Simulation of the water balance and the behaviour of methabenzthiazuron in lysimeters and a field trial with two simulation models. *Proc. IX Symposium Pesticide Chemistry, Piancenza, Italy*, 193-202.

Hopmans, J.W.; Vogel, T.; Koblik, P.D. (1992) X-ray tomography of soil water distribution in one-step outflow experiments. *Soil Science Society of America Journal*, **56**, 355-362.

Jarvis, N.J. (in press) Simulation of soil water dynamics and herbicide persistence in a silt loam soil using the MACRO model. *Modeling of Geo-Biosphere Processes*.

Peyton, R.L.; Haeffner, B.A.; Anderson, S.H.; Gantzer, C.J. (1992) Applying X-ray CT to measure macropore diameters in undisturbed soil cores. *Geoderma*, **53**, 329-340.

Rogasik, H.; Joschko, M.; Burnotte, J. (1994) Nutzung der Röntgen-Computertomographie zum Nachweis von Gefügeänderungen durch Mulchsaat. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft*, **73**, 111-114.

van Dam, J.C.; Sticker, J.N.M.; Drogers, P. (1990) From one-step to multi-step - Determination of soil hydraulic functions by outflow experiments. *Rapport 7. Landbouwuniversiteit Wageningen*.

van Genuchten, M.T.; Wagenet, R.J. (1989) Two-side/two-region models for pesticide transport and degradation: theoretical development and analytical solutions. *Soil Science Society of America Journal*, **53** (5), 1303-1310.

Vancloster, M.; Diels, J.; Viaene, P.; Feyen, J. (1993) The SWAT-modules: theory and input requirement. *Internal Report, Institute of Land and Water Management, Katholieke Universiteit Leuven, Belgium*.

Vereecken, H.; Maes, J.; Feyen, J. (1990) Estimating unsaturated hydraulic conductivity from easily measured soil properties. *Soil Science*, **149** (1), 1-12.

Warner, G.S.; Nieber, J.L.; Moore, I.D.; Geise, R.A. (1989) Characterizing macropores in soil by computed tomography. *Soil Science Society of America Journal*, **53** (3), 653-660.

THE USE OF IMMOBILISED ANTIBODY FRAGMENTS TO DETECT AND REMOVE ORGANIC POLLUTANTS IN THE ENVIRONMENT

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ABSTRACT

Antibody fragments to a range of organic pollutants have been cloned and expressed as bivalent dimers in *E. coli*. The antibody fragment have binding affinities approaching those of the parent monoclonals. Antibody fragments have been immobilised on beads and used to remove small organic molecules from aqueous environments.

INTRODUCTION

In recent years, new legislation and guidelines have placed increasing demands on water authorities and industry to clean-up chemical pollutants in our potable waters and groundwaters. Traditional methods for remediation are being improved but are likely, at best, to provide only a "first stage" of clean-up often leaving pollutants present in the environment at levels of ug/l (Harris 1992; Dommez 1986). Current European legislation and guidelines require a further 10-100 fold reduction in contamination so that no individual pollutant is present at levels higher than 0.1ug/l. The use of ozonation in conjunction with activated charcoal is effective at reducing pesticide levels (Edge 1994). However, it is expensive, both in capital and running costs, increasing the expense of water treatment by as much as 30% (Hart 1994). New cost effective technologies are therefore sought to fulfil these European objectives. What is required is a chelation methodology where the chelation matrix can be easily and cheaply recycled. Immobilised antibody fragments (antigen-binding proteins) could fulfil this need.

Antibodies are mammalian protein molecules produced *in vivo* and function as a main line of defence against foreign invaders including toxic chemicals (Fig 1). These antibodies bind strongly to their target and have the unique ability to remove antigens present in the blood at parts ng/l. If available outside the body, antibodies represent one of the few affinity compounds capable of binding organic compounds at concentrations at which they are present in the environment.

Monoclonal antibodies which bind smaller organic molecules have been produced by conjugating a small hapten to a larger carrier protein or by adsorbing the hapten onto an inert adjuvant (passive adsorption) (Shelton *et al*. 1994). Such techniques allow the production of monoclonal antibodies to small
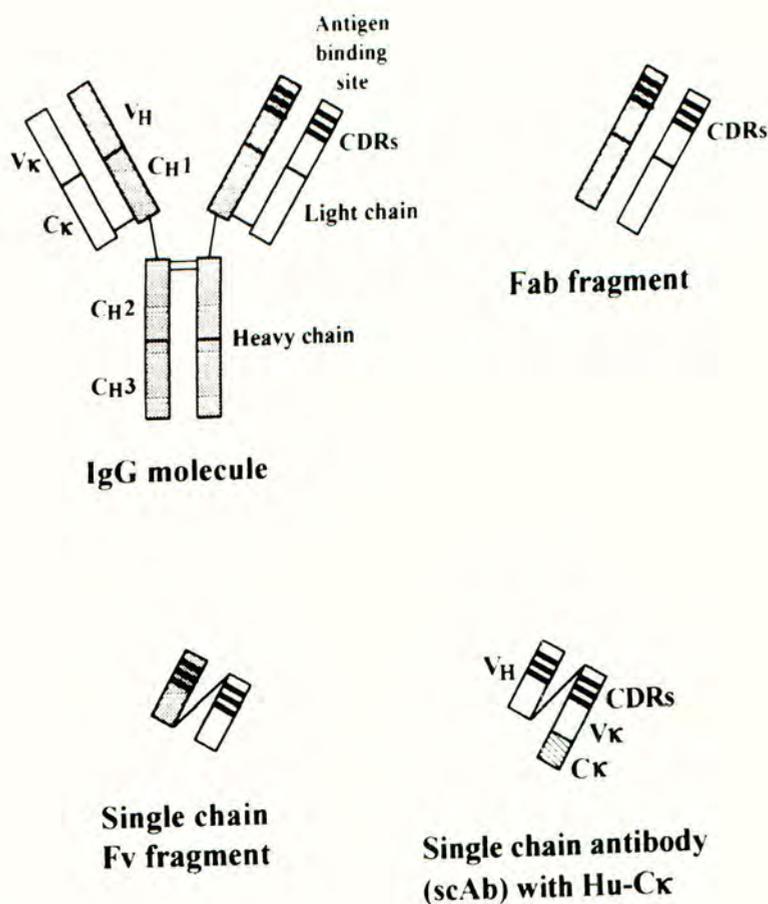
Figure 1. The relationship between naturally occurring antibodies and antibody fragments (antigen-binding proteins). The natural antibody is composed of two heavy (H) and two light (L or K) chains. The heavy and light chains are made up of constant (C) and variable (V) domains and describe the degree of variability in amino acid sequence. The V domains of both chains contain the antigen binding site. Three hypervariable regions or complementarity determining regions (CDRs), present within the V domains, form loops and are the major points of interaction between antigen and antibody. Subfragments containing these variable heavy (VH) and light (VL or VK) chains can be expressed and synthesized in *E .coli*. These antibody fragments can be produced in a number of forms and have been made more stable by linking them *via* a short flexible amino acid linker (ScFv). The single-chain antibody (ScAb) also includes a human constant region (Hu-CK) used to quantify and purify bacterially expressed fragments.

organic pollutants. However, widespread use of antibodies is limited by their large molecular weight and complex structure which requires production in mammalian cells at high cost. Antibody fragments (Fig 1a) representing as little as 20% of the intact molecule can be made in *E. coli* at acceptable costs and can be designed so that they retain the high binding affinity of the intact antibody (Bird and Walker 1991; Pluckthun 1991).

We have already shown in a model system that antibody fragments against bacterial cell surface epitopes can be made in *E. coli*, immobilised on a solid matrix and used to effectively remove bacteria from fluids (McGregor *et al.* 1994; Molloy *et al.* 1994). In this paper we will demonstrate that this technology can be extended to small organic molecules allowing pollutants to be identified and removed from aqueous environments. Commercially important pesticides (paraquat, triazine herbicides, diuron) have been used as antigens.

RESULTS

Polymerase chain reaction (PCR) rescue of variable heavy (VH) and variable light (VK) chain genes.

Using IgG specific primers (Orlandi *et al.* 1989), variable chain regions have been amplified from mouse cDNA obtained from monoclonal cell lines to paraquat, atrazine and diuron. The genes were cloned and sequenced in pUC18 and pGEMZ- vectors prior to subcloning into the expression vectors.

Expression and purification of single-chain antibody fragments.

The VH and VK chains were cloned into the single-chain vectors pPM1-His (Fig 2A) and pFLAG (Fig 2B) constructed in our laboratory as a modification of an earlier vector pDM1 (McGregor *et al.* 1994). pPM1-His is a novel construct contains a 14 amino acid linker (Chaudhary *et al.* 1990) and possesses a human kappa chain constant domain fused to the 3' end of the ScFv. For the purposes of this study we have described it as a single-chain antibody (ScAb) to distinguish it from a ScFv antibody. We have further modified this vector to include an amino-terminal affinity tag of six histidines inserted 3' of the constant light chain to facilitate purification. No effect on the production of the single-chain antibody (ScAb-His) was observed. Yields of around 0.3mg purified protein, from 1 litre of IPTG induced cultures, were obtained.
A second smaller construct, pFLAG, containing the same linker sequence but with the histidine tail and CK domain replaced with a short 8 amino acid affinity tag, has also been used to produce quantities of purified functional single-chain antibodies.

Antigen binding.

The relative antigen binding capacities of the different purified Scab-His and their corresponding parent monoclonal were compared. The monoclonals and their antibody fragments gave similar binding profiles and the fragments

Figure 2A

pPM1-His
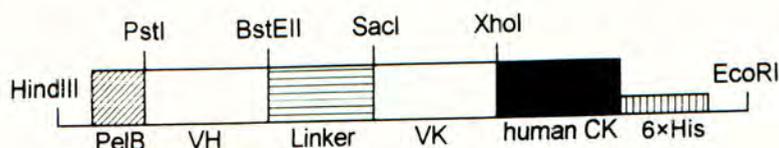
Figure 2B

pFLAG
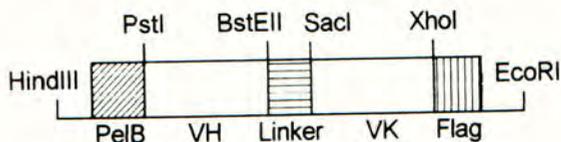
Figure 2. The arrangement of genes in the vector used for antibody fragment expression in *E. coli*. All coding regions were cloned down stream from a *lac* promoter in pUC 19. VH, VK and CK are the antibody fragment variable (V) and constant (C) domains respectively, Pb denotes a Pel B leader sequence which directs secretion of the peptide into the *E. coli* periplasm. The 6 x His denotes six histidine residues used to purify the protein by metal chelate affinity chromatography. The FLAG denotes an 8 amino acid tag used for affinity purification using a monoclonal antibody specific to this epitope. The linker (Chaudhary *et al.* 1990) and histidine tail were engineered into the vector as described previously (McGregor *et al.* 1994; Molloy *et al.* 1994).
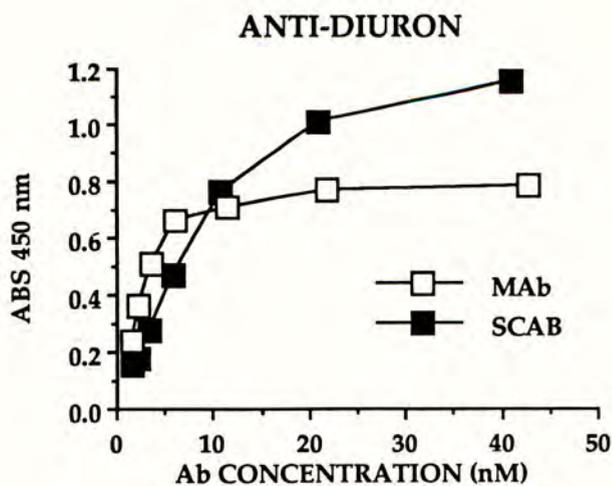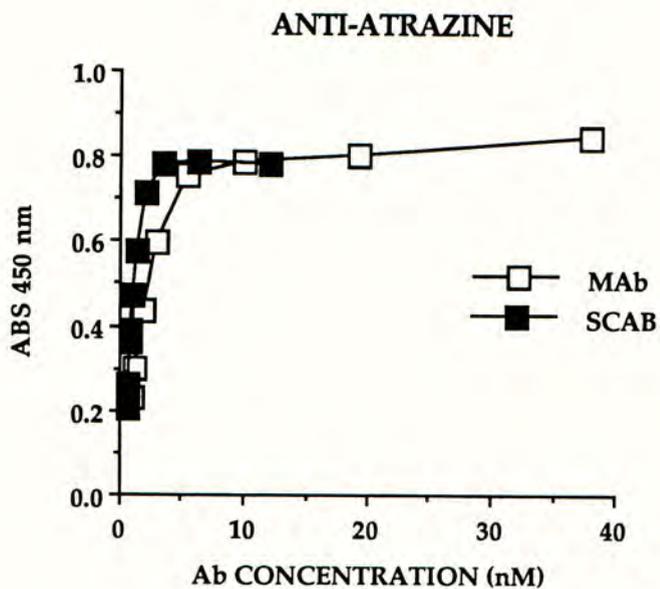
Figure 3. Comparison of the ability of equimolar amounts of monoclonal antibody (anti-atrazine and anti-diuron) and their corresponding antibody fragments to bind bovine serum albumin (BSA) conjugates (atrazine-BSA and diuron-BSA respectively). The binding of antibodies and antibody fragments was determined by ELISA.
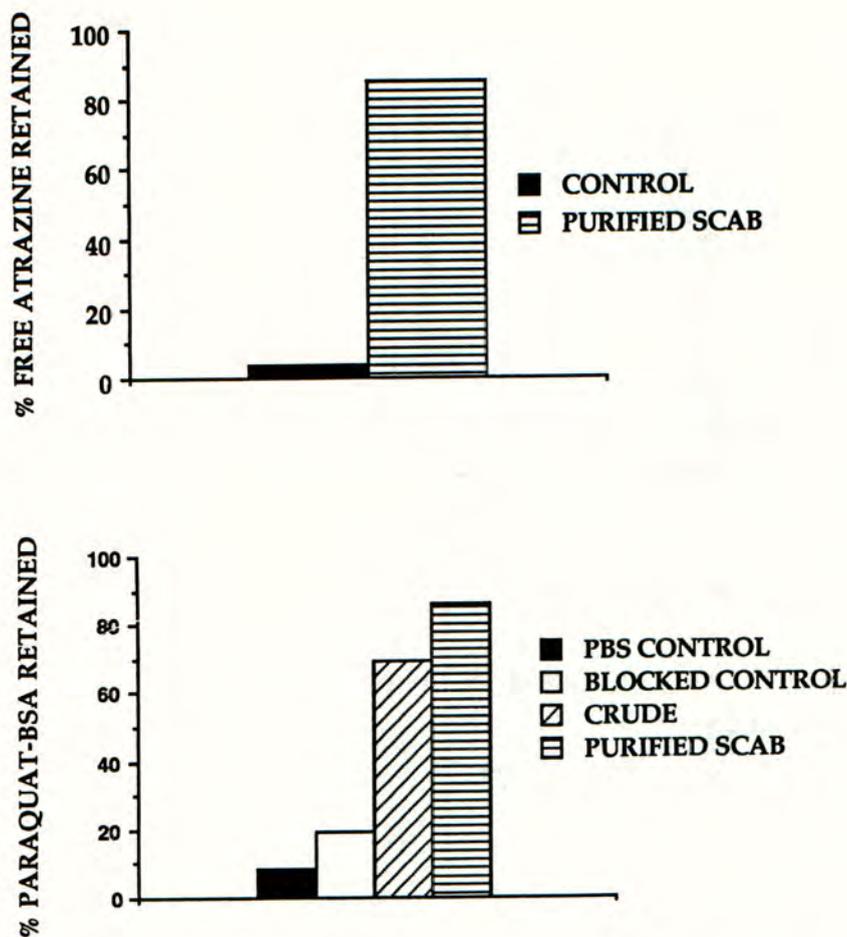
Figure 4. A. One millilitre of an aqueous solution of atrazine (120ug/l) was added to control beads blocked with 2% milk powder and beads coated with anti-atrazine single chain antibody (ScAb). The percentage of free atrazine bound by the beads after a 1h incubation (with gentle rotation) at 4°C was calculated by HPLC.

B. One millilitre of an aqueous solution solution of paraquat-BSA (1:10) conjugate (equivalent to 350ug/l paraquat) was added to four different bead preparations: uncoated control beads in phosphate buffered saline (PBS control), control beads that have been blocked with 2% milk powder (blocked control), and beads coated in a crude or purified preparation of anti-paraquat ScAb (crude or purified ScAb respectively). The percentage of paraquat-BSA conjugate bound to the beads after a 1h incubation (with gentle rotation) at 4°C was calculated by ELISA.

retained binding affinities, for target antigen, approaching those of the parent monoclonal (Fig 3). In general, the IC_{50} for free antigen was approximately 10 times higher for all antibody fragment formulations tested. Furthermore, in a direct competition ELISA approximately 10 times the concentration of antibody fragments were required to inhibit its corresponding monoclonal binding by 50%

Immunoaffinity separation and concentration of organic pollutants from aqueous environments.

Conditions for the immobilisation of ScAb-His protein have been optimised and preliminary experiments suggest that effective immunoabsobent columns can be constructed. Over 85% of paraquat and free atrazine, present at ug/l levels, can be removed from aqueous solutions (Fig 4). The reduced performance of the column coated with crude unpurified antibody fragments is probably the result of steric hindrance. The larger proteins present in bacterial lysate may cover the antibody fragments and prevent close association of the antigen and antibody binding sites. This situation can be mimicked by blocking beads coated with purified antibody fragments with BSA prior to use.

CONCLUSIONS

The project utilises the methods of genetic and protein engineering to produce fragments of antibodies (antigen binding sites), in the laboratory, which can detect and remove target pollutants present at low levels. We have cloned and expressed antibody fragments against the pesticides atrazine, paraquat, diuron and have developed systems for their purification from bacteria. In the near future, using molecular modelling and site directed mutagenesis, we hope to produce fragments with enhanced binding affinities and which are stable in non-physiological environments (Glockshuber *et al.* 1990; Brinkmann *et al.* 1993) including (organic solvents). We believe these will provide a new generation of detection/remediation systems which will meet legislative demands and are suitable for use in the field. Possible commercial applications include:

1. Antibody fragments immobilised on "dipsticks" as a sensitive , rapid and inexpensive method of detecting environmental pollutants.

2. In situations where water is highly contaminated "low tech" coarse filters could be used upstream of a specific immunoabsobent. This technology is also applicable to pesticide container decontamination.

In the longer term, we are currently producing our first transgenic plants capable of expressing antibody fragments to organic pollutants (Axis Genetics, Cambridge, UK). These plants could be useful as a cheap source of antibody fragments or as plants able to grow on polluted land to directly remove and immobilise pollutants present in the soil.

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REFERENCES

Bird, R.E. and Walker, B.W. (1991) Single chain antibody variable regions. *Trends in Biotechnology* **9**, 132-137.

Brinkmann, U., Reiter, Y., Jung, S.H., Lee, B. and Pastan I. (1993) A recombinant immunotoxin containing a disulfide-stabilized Fv fragment. *Proceedings of the National Academy of Science* **90**, 7538-7542.

Chaudhary, V.K., Batra, J.K., Gallo, M.G., Willingham, M.C., Fitzgerald, D.J. and Pastan, I. (1990) A rapid method of cloning functional variable-region antibody genes in *Escherichia coli* as single-chain immunotoxins. *Proceedings of the National Academy of Science* **87**, 1066-1070.

Dommez, M.P. (1986) Study of the discharge of certain dangerous substances into the aquatic environment and the best means for the reduction of water pollution from such discharges in application to the directive 76/464/CEE. EEC Report Number 85/B/6600/11/003/11/M

Edge, C. (1994) Anglian water's pesticide strategy. *Proceedings BICS International Conference on Pesticides and Water Quality*, London.

Glockshuber, R., Malia, M., Pfitzinger, I. and Pluckthun, A. (1990) A comparison of strategies to stabilize immunoglobulin Fv-fragments. *Biochemistry* **29**, 1362-1367.

Harris W.J. (1992) Commercial use of microbial inocula containing live genetically modified microorganisms in : The release of genetically modified micoorganisms Eds Stewart-Tull D.E. and Sussman M. Plenum Press, London 85-92.

Hart, J. (1994) Pesticide removal from water - treatment options and cost. *Proceedings BICS International Conference on Pesticide and Water Quality*, London

McGregor, D.P., Molloy, P.E., Cunningham, C. and Harris, W.J. (1994) Spontaneous assembly of bivalent single chain antibody fragments in *Escherichia coli*. *Molecular Immunology* **31**, 219-226.

Molloy, P., Brydon, L, Porter, A.J. and W.J. Harris (1994) Concentration and separation of bacteria using antibody fragments. *Journal of Applied Bacteriology* (in press).

Orlandi, R., Gussow, D.H., Jones, P.T. and G. Winter (1989) Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. *Proceedings of the National Academy of Science* **86**, 3833-3837.

Pluckthun, A. (1991) Antibody engineering: advances from the use of *Escherichia coli* expression systems. *Bio/technology* **9**, 545-551.

Shelton S.A., Graham, B.M., Porter, A.J. and W.J. Harris (1994) Detection and isolation of polychlorinated biphenyls. UK Patent Application No. 941-3436-8.

INFLUENCE OF CROP RESIDUES ON DEGRADATION, FORMATION OF BOUND
RESIDUES AND TRANSPORT OF METHABENZTHIAZURON IN SOIL.

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ABSTRACT

In order to study the effect of crop residues on degradation, formation of bound
residues, and translocation of methabenzthiazuron (MBT) in an orthic luvisol
(degraded loess soil) a lysimeter study (undisturbed soil cores, 1 m^2, 1,10 m deep)
and a complementary degradation study (0, 10, 20, 30 °C; 40 % of WHC) were
conducted. [Phenyl-U-^{14}C]MBT was applied preemergence to winter wheat ac-
cording to a rate of 3 kg AI/ha in November 1992. In the degradation study the
concentration of MBT was 0.47 mg AI/100 g dry soil. In both studies for one
treatment maize straw was mixed into the soil at rates equivalent to 9 t/ha
(lysimeter study) and 0.25, 0.5, 1.0, 1.5 and 3.0 g/100 g dry soil (degradation
study). The amendment of maize straw increased the microbial activity in the soil
as well as the mineralization, formation of its major metabolite and the formation
of bound residues of MBT. Probably due to the enhanced dissipation of MBT in
the soil the amount of MBT measured in the leachates of the lysimeter amended
with maize straw was reduced to about 50 % of the amount of MBT transported
with the leachate of the lysimeter without organic amendment.

INTRODUCTION

The stimulating effect of easily degradable organic substrates on the dissipation in soil
has been described first in the 1940s by Kries (1947) for 2,4-Dichlorophenoxyacetic acid.
Similar effects were found for triallate (Anderson, 1984), atrazine (Hance, 1973), metribuzin
(Pettygrove & Naylor, 1985) and MBT (Cheng *et al.*, 1975). In a laboratory scale study
Azam *et al.* (1988) found an increase of mineralization, metabolization and formation of
bound residues of MBT in a Pakistani soil after amendment of wheat straw. The present study
was conducted in order to investigate the influence of maize straw on the degradation and
translocation of MBT-residues under laboratory and field conditions.

MATERIAL AND METHODS

Lysimeter study

The lysimeters used were freedraining and contained undisturbed soil cores of 1 m^2
surface and 1.10 m depth (Steffens *et al.*, 1992). The soil cores had been taken from a field in
Merzenhausen, Rhineland, 10 km distance to the lysimeter facility. They had been sampled 5-
6 years before the experiment started and kept under good agricultural practice during stor-

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age under outdoor conditions. The soil used was an orthic luvisol (plough horizon (A_p): pH 7.2, 1.2 % C_{org}, 78.2 % silt, 15.4 % clay, 6,4 % sand). MBT (1-benzothiazol-2-yl-1,3-dimethyl-urea) is the AI in Tribunil® (WP, 70 % AI), an effective herbicide used in many cereal crops (Hack, 1969). In this study [phenyl-U-^{14}C]MBT was used as test substance having a specific radioactivity of 429.2 kBq/mg AI. Mittelstaedt *et al.* (1977) identified demethyl-MBT (1-methyl-1-(2-benzthiazolyl)urea as the major metabolite in the test soil. Winter wheat (variety Orestis) was sown on November 2, 1992 and harvested on July 20, 1993. MBT was applied at rates of 3 kg AI/ha (124 and 128 MBq) pre-em. to winter wheat on two lysimeters on November 10, 1992. On October 28, 1992 maize straw was mixed into the upper 5 cm of the soil of lysimeter **MBT+straw** at a rate equivalent to 9 t/ha simulating the rotational situation after maize straw harvest in fall. Lysimeter **MBT** stayed without organic amendment. Crop management was in agreement with good agricultural practice. The natural precipitation was supplemented to at least 800 mm/a according to the German guideline for lysimeter studies (BBA, 1990).

Degradation study

The degradation study was carried out using Erlenmeyer flasks with 100 g dry soil each (3 replicates) at 20 °C and 40 % of WHC. The adjusted concentration of MBT was 0.468 mg AI/100 g dry soil. In parallel to the lysimeter study maize straw was added to treatment **MBT+straw** at a rate of 1.5 g straw/100 g dry soil. Treatment **MBT** stayed without organic amendment. For both treatments additional flasks (without replicates) were kept at 0, 10, and 30 °C. For treatment **MBT+straw** additional flasks (3 replicates) were included with 0.25, 0.5, 1.0 and 3.0 g straw/100 g dry soil. Measurement of microbial activity in the soil was by reduction of dimethylsulfoxide (DMSO). Moist soil (10 g) were weighed into small Erlenmeyer flasks of known volumes (about 65 mL). After 10 min of incubation at 40 °C 2 mL DMSO, 10 % solution, were added and the sample kept at 40 °C for 3 h before the concentration of dimethylsulfid (DMS) was determined using gas chromatography (Alef and Kleiner, 1989, modified).

Sampling and analysis

Leachates from the lysimeter were taken at intervals of 2-3 weeks and analyzed for radioactivity. Aliquots of 1 l were extracted using solid phase (C-18 material) extraction. Extracts were submitted to radio-tlc with co-chromatography of the nonlabeled reference substances (MBT, demethyl-MBT). Soil samples were taken from the A_p-horizon of the lysimeter after the harvest of winter wheat, 255 days after application of MBT, and 10 and 40 days after application of MBT in the degradation study. The soil was subsequently desorbed with a simulated soil solution (0.01 M $CaCl_2$, 22h) and extracted with acetone (3h), ethylacetate (15h), and chloroform (1h). Residues of MBT were determined using radio-tlc. The soil residue was further extracted with 0.1 M $Na_4P_2O_7$ (three times 22h). Humic acids were precipitated by acidifying the solution to pH 2, centrifuged and redissolved in 0.1 M NaOH. Fulvic acids stayed soluble in the acidic supernatant. The nonextracted radiocarbon was considered to be bound to the humin.

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RESULTS AND DISCUSSION

The rapid degradation of the added maize straw increased the microbial activity in the soil (Figure 1) and promoted the turnover and mineralization of MBT (Figure 2). During the 40 days of incubation in the degradation experiment 1 % of the applied radioactivity was mineralized without and 5 % with addition of maize straw. In both, the lysimeter and degradation study, the amendment of maize straw led to a higher amount of demethyl-MBT measured in the soil (Table 1) indicating an enhanced degradation of MBT. However, the major path of dissipation was the additional formation of bound residues. After the harvest of winter wheat 36.7 % of the applied radioactivity were measured in bound residues for lysimeter **MBT** in comparison to 55.1 % for lysimeter **MBT+straw**. In the degradation experiment the difference between both treatments was even more pronounced. This can be explained by a) low temperatures (average temperature in 5 cm: 5 °C) during the first 5 months after application of MBT in the lysimeter study and b) length of incubation time. Within the bound residues relatively more ^{14}C was measured in fulvic and humic acids compared to the sorption or binding of ^{14}C to the humin fraction.
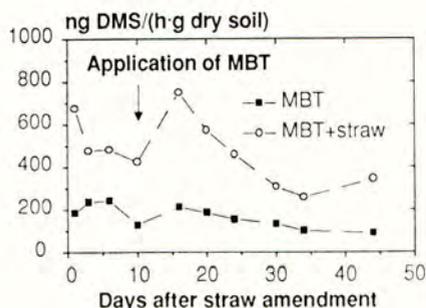
FIGURE 1. Microbial activity in degradation study treatments MBT and MBT+straw as measured by reduction of DMSO to DMS at 40 °C.
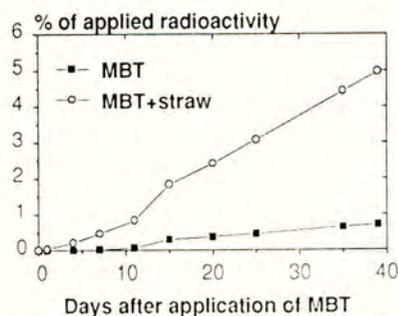
FIGURE 2. Accumulated amounts of mineralized radioactivity after application of MBT (degradation study).

TABLE 1. Soil concentrations of MBT and demethyl-MBT and radiocarbon in bound residue fractions after the harvest of winter wheat in the lysimeter experiment and at day 40 of the degradation experiment (20 °C, 40 % WHC). Percentage of applied radioactivity.

Amendment of maize straw	Lysimeter experiment		Degradation study	
	no	yes	no	yes
DAT	255		40	
MBT	28.8	8.9	56.9	7.7
Demethyl-MBT	0.7	4.0	2.8	6.1
Fulvic acids	4.2	10.6	6.4	15.8
Humic acids	4.5	13.9	5.5	35.9
Humin	28.0	30.6	16.4	31.1

The temperature is the major factor influencing the degradation of MBT in soil (Führ & Mittelstaedt, 1979). This could be confirmed with the degradation study (Figure 3). But the effect of temperature was different for the treatments **MBT** and **MBT+straw**. For treatment **MBT** the increase in dissipation and formation of bound residues is almost linear with temperature. After maize straw amendment the temperature effect on degradation is much more pronounced for temperatures below 20 °C. Between 20 and 30 °C an optimum was apparently reached for microbial degradation. Whereas only small amounts of demethyl-MBT were found for treatment **MBT,** about 7 % of the applied radioactivity represented the major metabolite for treatment **MBT+straw**.

treatment MBT   treatment MBT+straw
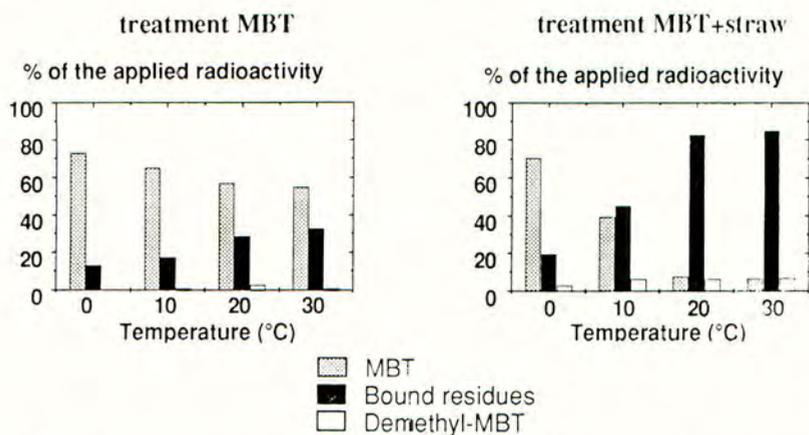
FIGURE 3. Soil concentrations of MBT and demethyl-MBT as well as radioactivity measured in bound residues after 40 days after application of MBT as influenced by the temperature of the experiment.
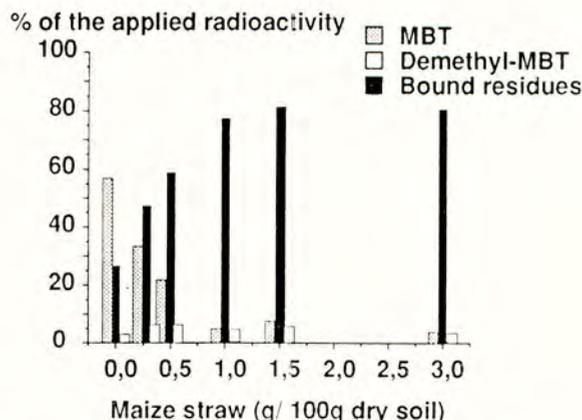
FIGURE 4. Concentrations of MBT and demethyl-MBT as well as radioactivity measured in bound residues 40 days after application of MBT as influenced by increasing amounts of added maize straw.

Small amounts of maize straw led to reduced concentrations of MBT in soil and an enhanced formation of bound residues (Figure 4). Assuming distribution in the upper 5 cm of topsoil and a bulk density of 1.2 kg/dm³ amounts of 0.25 and 0.5 g maize straw/100 g dry soil represent rates of 1.5 and 3.0 t maize straw/ha, respectively. The results indicate that a rate corresponding to 6.0 t maize straw/ha leads to an optimum for the microbial degradation of MBT. In practical agriculture, however, yields of maize straw can reach 8.0-10.0 t/ha.

The enhancing effects of plant residues on the degradation processes in soil was observed at a temperature of 10 °C (Figure 3) and by addition of small amounts of maize straw (Figure 4). Therefore it can be assumed that addition of plant residues and organic fertilizers also affects pesticide degradation in soil and contributes to the spatial variability of degradation under field conditions.
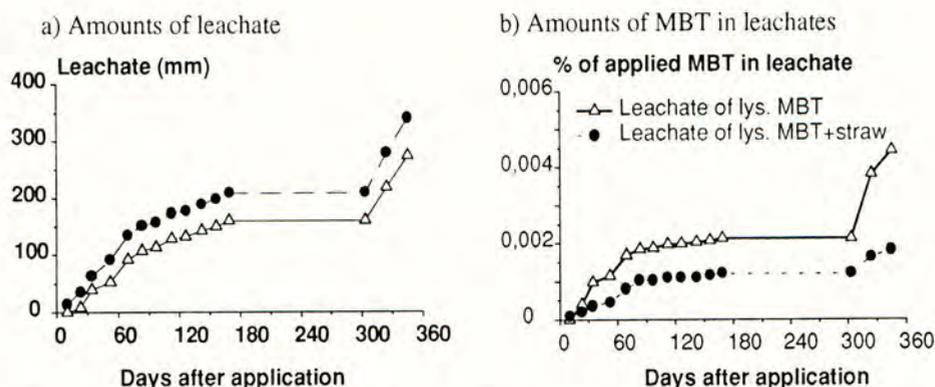
FIGURE 5. Accumulated amounts of leachate and residues of MBT in the leachates of lysimeters MBT and MBT+straw during the first year after application of MBT.

In lysimeter **MBT+straw** the total amount of MBT measured in the leachates only reached about 50 % of the corresponding amount measured in lysimeter **MBT** (Figure 5b). In lysimeter **MBT+straw** about 70 l more leachate were formed during the first year than in lysimeter **MBT** (Figure 5a). The average concentrations of MBT in the leachates were 0.02 µg MBT/l for lysimeter **MBT+straw** compared to 0.04 µg MBT/l for lysimeter **MBT**. The amendment of maize straw promoted the degradation of MBT in the soil and may therefore have limited the mobility of MBT through the soil profile.

CONCLUSIONS

The amendment of maize straw increased the mineralization and formation of the major metabolite and the formation of bound residues in soil. After organic amendment the temperature effect on dissipation was more pronounced below 20 °C compared to treatment without maize straw amendment and an apparent optimum for microbial degradation was reached between 20 and 30 °C. Relatively small amounts of maize straw affected the degradation of MBT in soil. Rates equivalent to 6.0 t maize straw/ha achieved optimal conditions for microbial degradation.

The enhancing effect of organic amendment on degradation of pesticide residues in soil may be of practical significance, because it was observed at temperatures below 20 °C and even for small amounts of maize straw.

The amendment of maize straw promoted the degradation of MBT in the soil. This may explain lower amounts of AI found in the leachates of lysimeter **MBT+straw** compared to lysimeter **MBT**.

ACKNOWLEDGMENTS

REFERENCES

Alef, K. & Kleiner, D. (1989) Rapid and sensitive determination of microbial activity in soils and in soil aggregates by dimethylsulfoxide reduction. *Biol. Fertil. Soils*, 8, 349-355.

Anderson, J. P. E. (1984) Herbicide degradation in soil: influence of microbial biomass. *Soil Biol. Biochem.*, 16, 5, 483-489.

Azam, F.; Führ, F. & Mittelstaedt, W. (1988) Fate of [carbonyl-^{14}C]methabenzthiazuron in an arid region soil - effect of organic amendment, soil disturbance and fumigation. *Plant and Soil*, 107, 149-158.

BBA (Biologische Bundesanstalt) (1990) *Richtlinien für die Zulassung von Pflanzenschutzmitteln in Zulassungsverfahren; Teil IV 4-3, Lysimeteruntersuchungen zur Verlagerung von Pflanzenschutzmitteln in den Untergrund.*

Cheng, H. H.; Führ, F & Mittelstaedt, W. (1975) Fate of methabenzthiazuron in the plant-soil system. In: Proc. 3rd IUPAC Congress of Pesticides Chemistry, *Environmental Quality and Safety*, Coulston, F & Korte, F. (Eds.), Vol. III, 271-276.

Führ, F. & Mittelstaedt, W. (1979) Effect of varying soil temperatures on the degradation of methabenzthiazuron, isocarbamid and metamitron. *Z. Pflanzenernähr. Bodenkd.*, 142, 657-668.

Hack, H. (1969) Tribunil, a new broad spectrum herbicide for cereal crops. *Pflanzenschutz-Nachrichten Bayer*, 22; 341-360.

Hance, R. J. (1987) Herbicide behaviour in the soil, with particular reference to the potential for ground water contamination. In: *Herbicides*, Hutson, D. H.; Roberts, T. R. (Eds), John Wiley & Son Ltd, 223-247.

Kries, O. H. (1947) Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter and time. *Bot. Gaz.*, 108, 510-525.

Mittelstaedt, W; Still, G. G.; Dürbeck, H. & Führ, F. (1977) Extraction and identification of the major metabolite of [carbonyl-^{14}C]methabenzthiazuron after degradation in the soil. *J. Agric. Food Chem.*, 25, 908-912.

Pettygrove, D. R. & Naylor, D. V. (1985) Metribuzin degradation kinetics in organically amended soil. *Weed Science*, 33, 267-270.

Steffens, W.; Mittelstaedt, W.; Stork, A. & F. Führ (1992) The lysimeter station at the institute of radioagronomie of the research centre Jülich GmbH (KFA), Germany. In: *Lysimeter Studies of the Fate of Pesticides in the Soil*, F. Führ and R. Hance (Eds), British Crop Protection Council, Monograph No. 53, 21-34.

SIMULATION OF HERBICIDE PERSISTENCE IN SOIL DURING AUTUMN AND WINTER

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ABSTRACT

Laboratory degradation and field dissipation studies with ethofumesate and chlorotoluron were conducted to characterize herbicide degradation during the autumn/winter period in an silty sand soil. Parameters for a simulation model were estimated from laboratory studies with incubation conditions of 1, 10, 20 and 30°C and 40%, 60% and 80% of the maximum water holding capacity. Regression analysis for parameter estimation *via* half-lives *vs.* direct estimation from laboratory concentrations and parameters (Arrhenius' activation energy, moisture dependence) *vs.* modified approach with optimum ranges of temperature and moisture conditions and microbial mortality rates are compared.

The degradation rates of both herbicides in the laboratory were increased with increasing temperature and moisture conditions, showing an optimum between 20 and 30°C, with a range of half-lives from 62-467 days for ethofumesate and 46-260 days for chlorotoluron. Comparing simulated with results from the field studies reveal that inclusion of low incubation temperatures improved the parameter estimation for autumn/winter periods. The modified approach of parameter estimation improved simulation results significantly as the field studies were conducted on bare soil.

INTRODUCTION

ANPROG, a module of the herbicide advisory system HERBASYS (Gottesbüren 1991; Gottesbüren *et al.* 1990; Pestemer *et al.* 1993), predicts herbicide persistence and effects of residues on succeeding crops, considering time and rate of application and site-specific soil and weather data. ANPROG simulates the degradation of herbicides in the soil (0-10 cm) using first-order kinetics according to the model PERSIST (Walker & Barnes 1981). This model has been validated with several herbicides and soils (e.g.

Walker & Barnes 1981, Gottesbüren *et al.* 1994). In general, the results of measured and predicted herbicide residues are in good agreement during the vegetation period. Sometimes herbicide dissipation is slightly underestimated. Our validation studies in the simulation period autumn/winter, however, reveal that the predicted dissipation rates during cold and wet periods are often highly overestimated.

The objective of this study was to improve the modeling for the period autumn/winter. Therefore, extended laboratory degradation studies with lower temperatures (1°C) and higher soil moisture contents (80% maximum water holding capacity; mwhc) were conducted for the herbicides chlorotoluron and ethofumesate to optimize the range for calculating the required parameters. Another emphasis was placed on a different approach to describe temperature and moisture dependence of herbicide degradation by a nonlinear function using a modified approach of Nörtersheuser (1993). First results are presented.

MATERIALS AND METHODS

Experimental design

The laboratory degradation studies were carried out with a soil (silty sand: 78.0% sand; 17.8% silt; 4.2% clay; 1.0% organic carbon; pH ($CaCl_2$) 5.9; mwhc 186 g H_2O kg^{-1} soil d.m.) of an agricultural site in Lower Saxony. Samples were taken from the top soil (0-10 cm).

The soil was sieved (2.5 mm) and stored at 4°C. After two weeks the soil was thoroughly mixed with the approved herbicide ethofumesate or chlorotoluron and moistened to 40%, 60%, 80% mwhc and incubated at 1, 10, 20 and 30°C in the dark. Ethofumesate and chlorotoluron concentration in soil (1.8, 1.5 mg kg^{-1} soil d.m. resp.) was calculated from a standard dose, assuming a soil density of 1.4 g cm^{-3} and a uniform distribution of the herbicide within the upper 10 cm of the soil. During incubation the water content was restored weekly. Samples were taken 1, 7, 14, 28, 56, 84 days after incorporation and stored at -18°C for residue analysis by hplc as described by Gottesbüren (1991).

The same site was used for the field experiments on bare soil. The herbicides used were commercial formulations of ethofumesate and chlorotoluron. On 1st October 1992 four replicate plots (6 m x 2.2 m) were sprayed with ethofumesate or chlorotoluron at standard doses. Immediately after spraying and 6, 18, 28, 54, 111 and 140 days after treatment eight soil samples were taken from each plot at random positions to a depth of 10 cm, thoroughly mixed and stored at -18°C for residue analysis.

Estimation of the parameters

i) Standardized method of parameter calculation for ANPROG

From the dissipation curves (first-order kinetics) the half-lives of the herbicides were determined for different incubation conditions (Timme *et al.* 1986). The dissipation parameters E_a (temperature dependence; equation 1a) and A, B (moisture dependence; equation 1b) were calculated according to Walker (1974) by linear regression procedure.

$$k = A_1 e^{-\frac{E_a}{RT}} \quad (1a) \qquad\qquad t_{1/2} = A_2 M^{-B} \quad (1b)$$

k = dissipation rate (d^{-1}); T = temperature (K); E_a = Arrhenius' activation energy $(kJ \, mol^{-1})$; R = gas constant $(kJ \, K^{-1} \, mol^{-1})$; $t_{1/2}$ = half-life (d); M = moisture content $(\% \, wt/wt)$; A_1, A_2, B = constants

ii) Approach for parameter estimation according to Nörtersheuser (1993)

The measured herbicide residues from incubation studies were also applied for fittings to equation (2) which is based on a combination of the O'NEILL-function and RICHTER-function.

$$k(T,\Theta) = k_{opt} \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right)^a e^{a \frac{T - T_{opt}}{T_{max} - T_{opt}}} \left(\frac{\Theta}{\Theta_{opt}} \right)^b e^{\left(1 - \left(\frac{\Theta}{\Theta_{opt}} \right)^b \right)} \quad (2)$$

T = temperature at time t $(°C)$; T_{max} = lethal temperature for micro-organisms $(45°C; fixed)$; T_{opt} = optimum temperature for maximum microbial activity $(°C)$; Θ = water content $(\% \, wt/wt)$; Θ_{opt} = optimum water content $(\% \, wt/wt)$; k = dissipation rate (d^{-1}); k_{opt} = dissipation rate at optimum temperature (d^{-1}); a, b = constants

Furthermore the rate of dissipation is determined by the concentration and a microbial mortality rate which evolve in time according to an ordinary differential equation (3a). Its solution leads to following concentration-time relation (3b):

$$\frac{dc}{dt} = k \, e^{-\alpha T t} \, c \quad (3a) \qquad\qquad c(t) = c_1 e^{\frac{k}{\alpha T} (e^{-\alpha T(t-1)} - 1)} \quad (3b)$$

c = herbicide concentration; c_1 = initial herbicide concentration; t = time (d); α = microbial mortality rate; k = dissipation rate (d^{-1}) (calculated by equation 2)

The parameters are estimated directly from degradation studies by minimizing the difference between measured and estimated concentrations with the method of least squares using the statistical programme package SAS.

RESULTS AND DISCUSSION

Laboratory experiments

From the laboratory degradation studies, the half-lives of the herbicides at different soil moisture and temperature conditions were calculated assuming first-order kinetics with good correlation. In general degradation was accelerated by increasing soil temperature with an optimum at 20°C as well as increasing water content. For both herbicides values of half-lives at low temperatures were very high (Table 1).

TABLE 1: Half-lives for ethofumesate and chlorotoluron in a silty sand under different incubation conditions

mwhc	ethofumesate $t_{1/2}$ [d]				chlorotoluron $t_{1/2}$ [d]			
	1°C	10°C	20°C	30°C	1°C	10°C	20°C	30°C
40%	467	-	105	-	260	102	57	-
60%	374	212	78	93	229	103	46	48
80%	192	146	62	-	207	99	46	-

The Arrhenius equation (1a) was fitted using the half-lives at different temperature ranges with 60% mwhc. For ethofumesate and chlorotoluron E_a-values are shown in Table 2. In some cases half-lives and soil temperature show a good positive correlation based on the Arrhenius equation. The calculation of E_a-values including higher temperature (30°C) was not satisfying as indicated by lower values of determination. The results show no differences between the herbicides.

TABLE 2: Dissipation parameters for temperature dependence (E_a) by 60% mwhc for ethofumesate and chlorotoluron in a silty sand with values of determination (r^2)

temperature	ethofumesate		chlorotoluron	
	E_a [kJ mol^{-1}]	r^2	E_a [kJ mol^{-1}]	r^2
1°C, 10°C, 20°C	55.2	0.979	56.4	1
1°C, 10°C, 20°C, 30°C	37.0	0.842	39.2	0.883
10°C, 20°C, 30°C	29.8	0.615	27.6	0.726

The values for the constants A_2 and B are presented in Table 3. Parameter B gives an estimate of the moisture dependence of degradation. Degradation of chlorotoluron (10°C) in this study was least influenced by the soil moisture level. This weak dependence is indicated by a low value of determination. Degradation of ethofumesate was more moisture-dependent.

TABLE 3: Dissipation parameters for moisture dependence (A, B) at 40, 60 and 80% mwhc for ethofumesate and chlorotoluron in a silty sand with values of determination (r^2)

temperature	ethofumesate			chlorotoluron		
	A_2	B	r^2	A_2	B	r^2
1°C	4870	1.233	0.863	559	0.368	0.998
10°C	-	-	-	112	0.043	0.426
20°C	983	1.189	0.929	119	0.365	0.848

Comparison of the parameter estimation methods was made by fitting equations (1) and (3) to the herbicide residues of all incubation variants for each herbicide. Therefor a transformed combination of equations (1a) and (1b) were used for calculation of a dissipation rate k_0 standardized at 28°C and $\Theta = 100\%$. Results are presented in Table 4. In general, fitting according to approach (ii) was much better as indicated by lower values of mean square. Only the estimation for parameter Θ_{opt} was not satisfying. The large confidence intervals (ethofumesate: -478 to 777; chlorotoluron: -60 to 250) indicate that the identification of parameter Θ_{opt} was not possible by approach (ii).

TABLE 4: Comparison of dissipation parameters estimated by different approaches for ethofumesate and chlorotoluron in a silty sand with values of mean square

parameter	estimates			
	approach (i)		approach (ii)	
	ethofumesate	chlorotoluron	ethofumesate	chlorotoluron
k_0 (i) / k_{opt} (ii)	0.013	0.023	0.019	0.028
T_{opt}	-	-	24.7	28.0
T_{max}	-	-	45 (fixed)	45 (fixed)
Θ_{opt}	-	-	150*	95*
α	-	-	0.00043	0.00043
E_a (i) / a (ii)	29.2	43.2	5.3	3.4
B (i) / b (ii)	0.69	0.18	0.94	0.69
Mean Square	0.00530	0.00305	0.00047	0.00069

* identification of the parameter was not possible

FIGURE 1: Comparison of observed and predicted persistence of ethofumesate and chlorotoluron in the field during autumn and winter using different approaches for parameter estimation
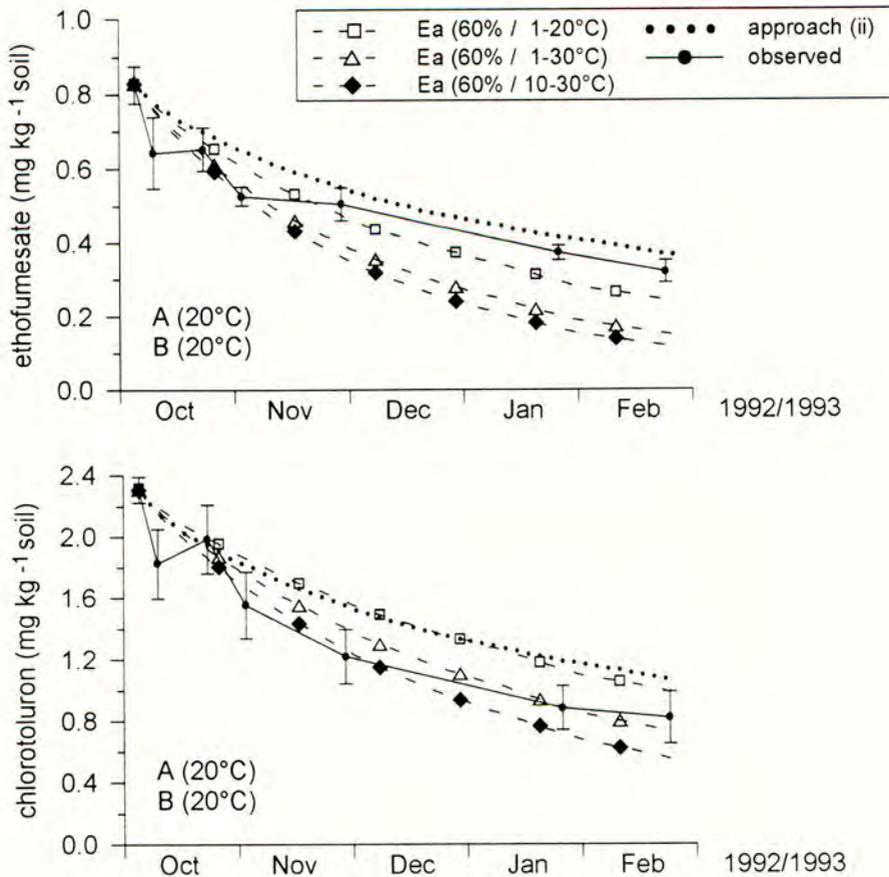
Prediction of persistence during autumn and winter by ANPROG

The results from the field studies are shown in Figure 1. The data show a rapid initial rate of loss followed by slower dissipation rate, which probably reflects the lower soil temperature from December onwards or microbial exhaustion of the bare soil. For approach (i) simulation curves produced by varying E_a-values in combination with A and B parameters calculated at 20°C are presented. Bold 'diamond' symbols illustrate the standard parameter set normally used for simulations during autumn/winter period. In both cases, this parameter combination resulted in the least accurate fit. With chlorotoluron, varying E_a-values led to an overestimation as well as an underestimation. For ethofumesate there was a general tendency to overestimate the rates of loss with all three parameter sets. But the results show, that using E_a-values, calculated from half-lives at low incubation temperatures, improve the fit to the measured residues during autumn and winter. As shown in Figure 1, predicted degradation curves based on approach (ii) gave a significant improved fit to the flat shape at the end of the measured residue curve. For chlorotoluron the predicted residues were consistently above those observed but the shape of the dissipation curve was better reflected by approach (ii). With ethofumesate, there was a good agreement, although the herbicide concentration was slightly overestimated.

REFERENCES

Gottesbüren, B. (1991) Konzeption, Entwicklung und Validierung des wissensbasierten Herbizid-Beratungssystems HERBASYS. Ph. D. Thesis, University of Hannover, Germany.

Gottesbüren, B.; Pestemer, W.; Wang, K.; Wischnewsky, M.-B.; Zhao, J. (1990) Concept, structure and validation of the expert system HERBASYS (Herbicide-Advisory System) for selection of herbicides, prognosis of persistence and effects on succeeding crops. *Symposium "Pesticides in soils and water: Current perspectives". BCPC Monograph*, **47**, 129-138.

Gottesbüren, B.; Beulke, S.; Heiermann, M.; Pestemer, W. (1994) Freiland- und Laborversuche zur Validierung von Prognosemodellen des Expertensystems HERBASYS zur Nachbauproblematik nach Herbizideinsatz. *Weed Research*, **34**, 63-78.

Nörtersheuser, P. (1993) Aufbau von Modellen zur Beschreibung des Verhaltens von Pflanzenschutzmitteln im Boden und Anwendung am Beispiel des Herbizids Quinmerac. Ph. D. Thesis, University of Braunschweig, Germany.

Pestemer, W.; Heiermann, M.; Günther, P. (1993) Implementation of Simulation Models into a Herbicide Advisory System (HERBASYS). *Modeling Geo-Biosphere Processes*, **2**, 129-141.

Timme, G.; Frehse, H.; Laska, V. (1986) Zur statistischen Interpretation und graphischen Darstellung des Abbauverhaltens von Pflanzenschutzmittel-Rückständen. II. *Pflanzenschutz-Nachrichten Bayer*, **39**, 188-204.

Walker, A. (1974) A simulaton model for prediction of herbicide persistence. *Journal of Environmental Quality*, **3**, 396-401.

Walker, A.; Barnes, A. (1981) Simulation of herbicide persistence in soil; a revised computer model. *Pesticide Science*, **12**, 123-132.

FACTORS CONTROLLING THE DEGRADATION OF 2,4-D FROM TOPSOIL TO SUBSOIL

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INTRODUCTION

Pesticide degradation is now seen as critical to the prevention of groundwater contamination, because it reduces the *total* amount of pesticide subject to leaching. Pesticide adsorption, however, does not reduce this amount. Rather adsorption reduces the *available* amount for leaching, which increases the residence time of pesticides in soil and permits more time for degradation. Likewise, the large extent of the subsoil region adds to the residence time of pesticides in soil, potentially permitting more time for degradation in subsoil than in topsoil. Subsoil degradation may therefore be more effective than has previously been assumed. Hence, there has been a growing interest in pesticide degradation below topsoil, and the associated subsurface microflora that mediate degradation.

The rate of pesticide degradation in soils is controlled by the microbial population size, its activity, and the availability of the pesticide to the population. Soil conditions also control this rate, such as moisture, temperature, pH and nutrient status (Anderson, 1984). Consequently, there is a need to understand the joint effects of these factors on pesticide degradation so that the risk of groundwater contamination can be assessed more accurately. This need includes understanding the effects of spatial variations of these factors in the field.

Previous investigations have focussed on pesticide degradation processes in the unsaturated zone of a sandy loam, as well as the size of the microbial biomass, activity and ability of the microbes to degrade pesticides (Lewis *et al.*, 1992).

The principal aim of the study here was then to examine some of the factors controlling the degradation of the pesticide 2,4-D in more detail, for both topsoil and subsoil using laboratory-based experiments. The factors examined include microbial biomass and pesticide availability, though other factors have been investigated (Lewis *et al.*, 1994). In particular, the study had three objectives:

(i) to examine whether degradation at different depths in the soil is predictable from measurements of controlling factors.

(ii) to investigate whether these controlling factors can be estimated accurately from soil properties.

(iii) to increase our knowledge of how these controlling factors and the associated degradation vary spatially across a field.

MATERIALS AND METHODS

Soil Collection

Soil was sampled at a field site at Frensham, Surrey, comprising a sandy loam not subject to pesticide applications for 10 years. The site was split into four blocks of 36 m x 36 m. In each block, 6 sampling points were chosen using a nested design. Distances between samples increased according to a geometric series to allow a large range of spatial scales to be covered in a single analysis (Webster and Oliver, 1990). The samples were taken down to 1 m using a Giddings hydraulic soil sampler with an acetate liner. The cores were divided into 25-cm depth increments for analysis. A hierarchical analysis of variance was then be used to understand the implications of spatial variations in degradation and the factors affecting degradation.

Measurements

Pesticide degradation was assessed by monitoring the mineralisation of [$ring$-U-^{14}C]2,4-dichlorophenoxyacetic acid (2,4-D) to $^{14}CO_2$ in a closed system. The availability of 2,4-D was determined by measuring the adsorption properties of the radiolabelled compound. Microbial biomass was determined by substrate induced respiration (Anderson and Domsch, 1978). Each soil sample was also analyzed for cation exchange capacity, organic matter, pH, particle size distribution, moisture retention (0.33 and 15 bar), and moisture content.

RESULTS AND DISCUSSION

The results from this study will be analyzed and discussed as follows:

(i) Degradation at Different Soil Depths and the Factors Controlling Degradation

The quantitative relationship between the amount of pesticide degraded at different soil depths and the factors controlling degradation (biomass and pesticide availability) will be determined, to examine whether pesticide degradation at different depths in the soil profile is predictable from measurements of controlling factors.

(ii) Correlations of Factors Controlling Degradation with Soil Properties

Correlations between controlling factors (microbial biomass and pesticide availability) with soil properties will be determined, to assess the plausibility of using soil properties for degradation predictions instead of the controlling factors. The measurement of soil properties is generally easier and cheaper than the controlling factors, particularly where large numbers of measurements are needed.

(iii) The Spatial Variability of Controlling Factors and Degradation

The variation of controlling factors and the associated degradation will be assessed across the sampling area, to allow a general link between laboratory-based experiments and field conditions, but particularly to assess the potential implications of such variations on: (a) the fluxes of pesticides to groundwater, and (b) field-based experimental design.

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REFERENCES

Anderson J.P.E. (1984) Herbicide Degradation in soil: influence of Microbial Biomass. *Soil Biology and Biochemistry* **16**, 483-489.

Anderson J.P.E. and Domsch K.H. (1978) A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biology and Biochemistry* **10**, 215-221.

Lewis K.J., Lewis F.J., Takagi K., Anderson J.P.E., Dictor M-C., and Soulas G. (1992) Microbial Diversity and Activity in Subsoils: Characterisation of Microflora from the Unsaturated Zone. In: *Proceedings International Symposium on Environmental Aspects of Pesticide Microbiology*. Sigtuna, Sweden. pp 270-277.

Lewis K.J., Lewis F.J. and Dyson J.S. (1994) Potential Activity of Microorganisms in the Unsaturated Zone of a Sandy Soil. In: *Proceedings Fifth International Workshop on Environmental Behaviour of Pesticides and Regulatory Aspects*, Brussels, Belgium. p 73

McNabb J.F. and Dunlap W.J. (1975) Subsurface biological activity in relation to groundwater pollution. *Groundwater* **13**, 33-34.

Webster R. and Oliver M. A. (1990) *Statistical Methods in Soil and Land Resource Survey*. Oxford University Press.