SESSION 6

HERBICIDE BEHAVIOUR IN SOIL: I

CHAIRMAN DR I. J. GRAHAM-BRYCE

SESSION ORGANISERS DR K. S. KILLHAM
DR A. WALKER

INTERACTIONS OF PESTICIDES WITH THE SOIL MICROBIAL BIOMASS

M.P. GREAVES

Institute of Arable Crops Research, Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS18 9AF, U.K.

ABSTRACT

The present status of measuring the side-effects of pesticides on the soil microflora is evaluated. It is concluded that there are drawbacks arising partly from the methods themselves, but mainly from the variability inherent in soil.

Some alternative approaches, emphasising soil biomass measurements are discussed. These offer considerable potential as sensitive indicators of response to stress but still suffer from the problems associated with the use of soil.

INTRODUCTION

Modern agriculture is dependent upon the use of biologically active chemicals to maintain yields and product quality. This has resulted in the general perception that the environment may be at risk through exposure to these chemicals. Consequently, there has been much research to try to quantify and evaluate the degree of risk involved. The soil microflora is well recognized as being essential in cycling organic matter and plant nutrients. It is not surprising, therefore, that much attention has focussed on interactions between the microflora and pesticides. Herbicides have been examined in particular detail as they are used, and so enter soil, in much greater quantities than any other pesticide. Recent development of compounds with very high, broad-spectrum, activity has heightened awareness of the possibility of affecting the soil microflora. The assertion that such compounds are environmentally safer than chemicals with lower activity because 'so much less chemical is used' does not stand critical examination. Environmental risk accrues from the product of dose and activity and is not solely dose related.

As a result of this awareness of hazard, many registration authorities have requirements which oblige companies to provide data demonstrating lack of critical effects of their products on the soil microflora. Most of these requirements, certainly in Europe, are based on the recommendations published by Greaves *et al.* (1980) and subsequently revised by Somerville *et al.* (1986). These recommendations refer primarily to assessing effects on soil respiration and ammonification and nitrification. These generally are accepted as the most appropriate means of detecting pesticide-induced stresses on the soil microflora which are presently available.

This acceptance of their status does not imply that they are intrinsically good methods. Indeed, most researchers will readily agree that they have grave shortcomings. It is disturbing that, despite their limitations, these methods have been advocated for at least thirty years with little change. More disturbing is the lack of serious contenders for their place as generally recommended methods of detecting perturbation in the soil microflora. This despite the great efforts by microbial ecologists to devise means of analysing the composition and behaviour of soil microbial populations. It also contrasts, markedly, with the advances being made in

agricultural microbial biotechnology, dependent as that is on a full and detailed understanding of how micro-organisms behave in different environments.

MICROBIAL INTERACTIONS WITH HERBICIDES

The literature presents thousands of papers reporting effects of herbicides and other pesticides on different aspects of the composition and activity of the soil microbial biomass. The accumulated data purports to show that different herbicides can and do affect the soil microflora in many ways, some being interpreted as harmful. It is clear, however, that the situation is extremely confused. Although many papers cannot be compared, through lack of essential information or choice of inadequate techniques, it is still inescapable that many herbicides cause widely differing effects, when examined in very similar experiments by different investigators. This is exemplified by data published by Anderson (1987) and produced in a ring test, by five laboratories, of the effect of mercuric chloride on carbon mineralization in soil (Table 1).

TABLE 1

Ring test by 5 laboratories (A-E) to examine inhibition of carbon mineralization in a sandy loam soil by mercuric chloride. (Data from Anderson, 1987)

Time (days)	Mineralization (% Control)					Mean ± SD	% Deviation from mean
	A	B	C	D	E		
1	96	93	77	92	98	91±8	8
7	63	42	21	57	80	53±22	42
14	55	28	15	40	58	39±18	46
28	45	29	7	19	38	28±15	53

These data show clearly that each laboratory was producing significantly different results, especially as incubations progressed. Anderson (1987) has also demonstrated that repeat experiments in the same laboratory can be quite reproducible, thus emphasising the small differences in technique between laboratories as a major source of variation in results.

Obviously, there are many other reasons why data from different experiments are not comparable. It is not my purpose to review these but, taken as a whole, the observed variability does lead to the conclusion that, at least with the currently recommended techniques, it is not possible to determine unequivocally if a herbicide will harm the soil microflora.

This conclusion, which has been drawn by some researchers (including the author) for many years, is confirmed by the recent decision of the Dutch authorities (van Doorn, 1987) to follow the U.S. Environmental Protection Agency and withdraw their requirement for soil microflora data, except for data on soil nitrification for soil-incorporated chemicals. Their view is that "nowhere is it spelt out precisely what is relevant information on the side-effects on soil microflora and how this information should be weighed when judging a pesticide's admissibility".

NEW APPROACHES

In view of the acknowledged flaws in the recommended methods of evaluating pesticide-induced stresses on the soil microflora, are there alternative approaches which might be adopted? Greaves (1987) has outlined some possibilities, though these simple approaches have not met with general approval. These include an automated miniaturized toxicity test (Cooper *et al.*, 1978), using large numbers (hundreds) of pure cultures of micro-organisms isolated from soil. The technique has been used for more than seventy herbicides and the results, generally showing no untoward toxicity, even at doses ten times higher than that expected in soil, confirmed by conventional side-effect testing. Greaves (1987) advocated this as a Tier 1 test which could preclude the need for expensive further tests for many chemicals which show no inherent toxicity.

If the view that the only acceptable way of assessing side-effects is to work with soil itself prevails, despite the problems of variability and lack of reproducibility, the available alternative methods are few and, as yet, relatively untried. Determination of the response of the microbial biomass itself seems most hopeful, though there is the inevitable disagreement as to choice of exact method.

The favoured method of measuring soil microbial biomass is that developed by Jenkinson and Powlson (1976) and based on fumigation or some variant of it (e.g. Lynch and Panting, 1980). Anderson and Domsch (1978) developed a physiological or respiratory method which may apply to a wider range of soils and conditions (e.g. pH and organic matter content) than that of Jenkinson and Powlson (1976). Sparling (1981) advocates the use of micro-calorimetry. A range of further approaches has been developed, all claiming some advantages. Thus, ATP determintion (Oades and Jenkinson, 1979; Jenkinson *et al.*, 1979) can give good correlation with biomass and can give early indications of change in microbial function. Following this work, Brookes *et al.* (1983) and Brookes *et al.* (1987) have used measures of the adenylate energy charge in soil to indicate the metabolically active biomass. Van de Werf and Verstraete (1987) have monitored, in a continuous respirometer, the oxygen uptake of soils. The data obtained is used to derive simultaneous estimates of biomass and a number of microbial growth kinetic parameters. Their results led them to conclude that soil biomass is a sufficiently sensitive indicator of stress in the soil to identify it as a parameter with potential value for microbial ecologists.

A particularly interesting approach is that developed by Killham (1985), following the earlier finding, by Killham and Firestone (1984), that soil micro-organisms divert more energy from growth into maintenance of cell integrity as stress increases. This leads to the expectation that an increasing proportion of C-uptake will be respired as stress increases. Thus, changes in the ratio of respired C to biomass C should be a good indicator of response to pesticide-induced stress. Indeed, Killham's (1985) data confirm this possibility, showing that the ratio was a sensitive indicator of stress from simulated acid rain containing heavy metals.

Killham (1985) points out that marked discrepancy between the effects of environmental stress on the ratio and on respiration raises "fundamental questions concerning our future approach to assessing the impact of environmental stresses and concerning interpretation of previous studies which relied solely on respiration "..... as determinants of stress-induced microbial changes". In some instances, growth of the microbial biomass

could be stopped entirely while respiration data might show no adverse impact of the environmental stress.

I have no doubt that this approach of Killham (1985) has great potential as a means of detecting pesticide side-effects. It remains to evaluate it with a range of chemicals and I hope this can be done as a matter of urgency.

APPLICATION OF BIOMASS MEASUREMENTS

Aside from the disagreements with regard to detailed choice of method, it is clear that all the methods suffer from common serious drawbacks, at least in the context of their application to determining pesticide side-effects in soil. These, essentially, mainly stem from the nature of soil itself. Soil is a living, dynamic system which is frequently likened to animal or plant tissue in attempts to present its complexity of function. As such, it responds not only to the pesticide under test, but also to all the stresses placed upon it during preparation for the test. Of these, soil storage is perhaps the foremost. Anderson (1987) and van de Werf and Verstraete (1987) have highlighted the loss of biomass occurring during soil storage (Table 2). So long as adequate experimental controls are included,

TABLE 2

Changes in microbial biomass during storage of a parabrown soil at different temperatures. (Data from Anderson, 1987)

Time (days)	Biomass (mg microbial C kg^{-1} dry soil) at 2'C	17'C	27'C
0	296	296	296
7	308	280	248
28	272	228	168
70 (% Loss)	244(18)	188(36)	112(62)

this loss of biomass should not affect the evaluation of the effect of pesticide treatment. However, significant (up to 50%) losses can also occur (Table 3) during the incubation of unamended soils (Anderson, 1987).

TABLE 3

Changes in microbial biomass of a fresh parabrown soil during incubation. (Data from Anderson, 1987)

Time	Biomass (mg microbial C kg^{-1} dry soil)	
(days)	Unamended soil	Soil + Glucose
0	390	390
7	380	516
28	316	527
70	190	630

They are accompanied by changes in the quality of the surviving microflora which may result in more or less susceptibility to the pesticide than would

occur in the field.

The lesson to be learned is clear. For any method to be fully useful for measuring microbial parameters in soil, particular care must be taken to ensure that the soil used is as representative of that in the field as possible. Preferably, it should be freshly collected but, if storage cannot be avoided, this should be at 2-4'C for a maximum of three months and drying should be avoided without limiting gas exchange. Even if these conditions are satisfied, all methods will still only give data which is comparative within a single experiment, though, with careful experimentation, comparisons between experiments in one laboratory may be acceptable. Other comparisons may not be valid owing to the high inherent variability in soils and their microbial components (Cook and Greaves, 1987).

CONCLUSIONS

There is little doubt that currently recommended methods of assessing interactions of pesticides with the soil microbial biomass are seriously disadvantaged. Despite this, there is a clear need to undertake some testing of chemicals before they are released into the environment, if only to allay public demands that some protective measures are taken.

Although relatively little advance has been made in our knowledge of how to test effectively for hazardous side-effects, recent work has indicated promising lines of research. In particular, analysis of microbial biomass in terms of its metabolic efficienty seems to be potentially most rewarding. Unfortunately, none of the newer methods has yet been fully evaluated with pesticides and this must be rectified urgently. Recent trends in research funding, both in the U.K. and elsehwere, have resulted in a significant decline in research in this area. It is a matter of concern that this is so at a time when there is a marked tendency for the agrochemical industry to produce chemicals of considerably greater potency, albeit to be used at lower doses, than previously. The lack of any known significant side-effects on the soil microflora, in practice, cannot be taken as evidence that effects will never occur. Indeed, it could be argued, boosted by increasing chemical potency, that we have been lucky and side-effects are imminent. If this risk is to be avoided, we must maintain research to ensure that valid, effective methods of predicting risks to the microflora from pesticides are made available.

REFERENCES

Anderson, J.P.E. (1987) Handling and storage of soils for pesticide experiments. In Pesticide effects on soil microflora, (Eds. L. Somerville and M.P. Greaves), London, Taylor and Francis, 45-60.

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

Brookes, P.C., Tate, K.R. and Jenkinson, D.S. (1983) The adenylate energy charge of the soil microbial biomass. Soil Biology and Biochemistry, 15, 9-16.

Brookes, P.C., Newcombe, A.D. and Jenkinson, D.S. (1987) Adenylate energy charge measurements in soil. Soil Biology and Biochemistry, 19, 211-217.

Cook, K.A. and Greaves, M.P. (1987) Natural variability in microbial activities. In Pesticide effects on soil microflora (Eds. L. Somerville and M.P. Greaves), London, Taylor and Francis, 15-43.

Cooper, S.L., Wingfield, G.I., Lawley, R. and Greaves, M.P. (1978) Miniaturized methods for testing the toxicity of pesticides to micro-organisms. Weed Research, 18, 105-107.

Greaves, M.P., Poole, N.J., Domsch, K.H., Jagnow, G. and Verstraete, W. (1980) Recommended tests for assessing the impact of pesticides on the soil microflora. Technical Report Agriculturl Research Council Weed Research Organization, 59, 15pp.

Greaves, M.P. (1987) Side-effects testing: an alternative approach. In Pesticide effects on the soil microflora (Eds. L. Somerville and M.P. Greaves), London, Taylor and Francis, 183-190.

Jenkinson, D.S. and Powlson, D.S. (1976) The effects of biocidal treatments on metbolism in soil - V. A method for measuring soil biomass. Soil Biology and Biochemistry, 11, 521-527.

Killham, K. (1985) A physiological determination of the impact of environmental stress on the activity of microbial biomass. Environmental Pollution (Series A), 38, 283-294.

Killham, K. and Firestone, M.K. (1984) Proline transport increases growth efficiency in salt-stressed Streptomyces griseus. Applied and Environmental Microbiology, 48, 239-241.

Lynch, J.M. and Panting, L.M. (1980) Variations in the size of the soil biomass. Soil Biology and Biochemistry, 12, 547-550.

Oades, J.M. and Jenkinson, D.S. (1979) Adenosine triphosphate content of the soil microbial biomass. Soil Biology and Biochemistry, 11, 201-204.

Somerville, L., Greaves, M.P., Domsch, K.H., Verstraete, W., Poole, N.J., van Dijk, H. and Anderson, J.P.E. (1986) Recommended laboratory tests for assessing the side-effects of pesticides on soil microflora.

Sparling, G.P. (1981) Microcalorimetry and other methods to assess biomass and activity in soil. Soil Biology and Biochemistry, 13, 93-98.

Van Doorn, A.M. (1987) Pesticide side-effects: regulations in the Netherlands. In Pesticide effects on soil microflora (Eds. L. Somerville and M.P. Greaves), London, Taylor and Francis, 191-196.

Van der Werf, H. and Verstraete, W. (1987) Estimation of microbial biomass by stimulation of respiration. In Pesticide effects on soil microflora (Eds. L. Somerville and M.P. Greaves), London, Taylor and Francis, 147-167.

THE INFLUENCE OF THE HERBICIDE TRIFLURALIN, ALONE AND IN THE PRESENCE OF SIMULATED ACID RAIN, ON THE ALGAE AND CYANOBACTERIA OF A SANDY LOAM SOIL

A.E. PIPE

Lakeland College, Vermilion, Alberta, Canada

ABSTRACT

Field plots were established in a sandy loam soil. The plots were treated with trifluralin to give concentrations of 0, 1 and 2 mg/kg in the top 1 cm of soil. One week later, and weekly throughout the growing season, plots at each herbicide level were treated with simulated acid rain at pH 4.5 and 3.5 respectively. The response of the soil algae and Cyanobacteria was monitored for 12 months using a slide colonization technique. The diatom Hantzschia and the Cyanobacterium Oscillatoria formed greater than 95% of the flora colonizing slides from control plots. Growth of Hantzschia was inhibited by both 1 and 2 mg/kg trifluralin, particularly when these treatments were followed by application of simulated acid rain. Inhibition was noted for up to 12 months after herbicide treatment. It is suggested that the acid may have facilitated the action of trifluralin on the algal cells by weakening cell membranes. It may also have affected degradation and adsorption characteristics of the herbicide. Oscillatoria was less sensitive to the trifluralin and simulated acid rain. This may be attributable to its clumped growth form and to the presence of mucilaginous sheaths covering its filaments. The data suggest a potential threat to populations of soil algae and Cyanobacteria in areas in which acid rain falls on soils containing trifluralin residues.

INTRODUCTION

The soil algae and Cyanobacteria have recently begun to be recognized for their role in the maintenance of soil fertility (Fogg et al., 1973, Pipe & Shubert, 1984). Concomitantly, the potential threat to soil fertility through interference with the activities of these organisms by agricultural practices such as herbicide use has been examined (McCann & Cullimore, 1979). Much of this work has been conducted under in vitro conditions (Pipe & Shubert, 1984). This is unfortunate, since the complex behaviour of herbicides in the soil renders prediction of field responses from in vitro studies virtually impossible.

In the present paper, a field study on the influence of the herbicide trifluralin (2,6-dinitro-NN-dipropyl-4-trifluoromethylaniline) on the algae and Cyanobacteria of a sandy loam soil is described. In addition to the application of trifluralin, the soil was also treated with simulated acid rain, in an attempt to elucidate any combined effect of these agents. The response of the algae and Cyanobacteria was determined by

means of an implanted slide technique (Pipe & Cullimore, 1980), in which microscope slides are placed vertically in the soil and become colonized by the microorganisms therein. On removal of the slides from the soil, the colonizing organisms can be examined microscopically, identified and enumerated.

MATERIALS AND METHODS

Establishment of field plots

Field plots (20 x 20 cm) were established in a sandy loam soil (clay 19%, sand 63%, silt 18%, organic matter 12%, water-holding capacity 53%, pH 7.5) shortly after spring thaw. Microscope slides, back-to-back in pairs, were implanted vertically into each plot (3 pairs per plot) such that the top 1 cm of each pair remained above the soil surface.

Application of trifluralin

Plots were treated with trifluralin in the middle of June (3 weeks after slide implantation), to give concentrations of 0, 1 and 2 mg/kg in the top 1 cm of soil (9 plots per treatment level). The herbicide was applied in a water carrier using a spray bottle with the nozzle adjusted to deliver a fine spray evenly over the surface of each plot. This was followed by incorporation by careful mixing of the soil to a depth of 1 cm, thereby approximating the application method recommended for trifluralin (Anderson, 1977).

Application of simulated acid rain

One week after trifluralin application, and weekly throughout the growing season, 3 plots at each herbicide level were treated with simulated acid rain at pH 4.5 and 3.5 respectively. The treatment solution consisted of de-ionized water containing (mg/l): SO_4^{2-}, 2.1; NO_3^-, 0.95; Cl^-, 0.29; NH_4^+, 0.48; Na^+, 0.34; K^+, 0.25; Ca^{2+}, 1.15; Mg^{2+}, 0.19 (average ionic composition of natural rainfall in the study area). Sulphuric acid was added to yield the specified pH values. The remaining 3 plots at each herbicide treatment level received the above solution adjusted to the average pH of natural rainfall in the area (5.6). All treatments were conducted using a spray bottle with the nozzle adjusted to deliver a fine spray evenly over the surface of each plot. The volume of treatment solution (200 ml per plot) was sufficient to moisten dry soil to water-holding capacity to a depth of 1 cm. Total natural rainfall during the simulated acid rain treatment period was 22 cm.

Recovery and examination of slides

Microscope slides were removed from the plots 2, 3 and 12 months after treatment with trifluralin (1 pair of slides from each plot on each occasion). They were prepared for microscopic examination as described by Pipe & Cullimore (1980), using molten 2% water agar to form a coating over the soil particles and microorganisms. The extent of colonization of the slides by algae and Cyanobacteria was determined by identifying and counting all cells observed on the part of each slide corresponding to the top 1 cm of the soil. Mean values were calculated for each separate treatment (6 slides per treatment). Significance of the data was determined by means of the Mann-Whitney test (Snedecor & Cochran, 1967).

Determination of soil pH

Soil samples were collected for the determination of pH each week immediately before treatment of plots with simulated acid rain, and at the conclusion of the experiment, 12 months after herbicide treatment. These samples were taken from a set of 27 plots set up and treated in exactly the same way as were those containing implanted slides. By using separate plots for pH determination, interference with the soil in the implanted slide plots was avoided. The soil pH was determined by the method of Pramer & Schmidt (1964).

RESULTS

A variety of algae and Cyanobacteria were observed colonizing the slides following their recovery from the soil plots. These were: the diatoms Hantzschia, Navicula and Pinnularia; the green algae Chlorella, Chlorococcum, Hormidium and Spongiochloris; and the Cyanobacteria Anabaena and Oscillatoria. Attention in this paper will be devoted to Hantzschia and Oscillatoria, which together formed greater than 95% of the flora colonizing slides from control plots. Hantzschia occurred consistently on all slides from control plots, and Oscillatoria on all except those recovered 2 months after herbicide treatment. Data presented for both of these organisms are therefore limited to the 3 and 12 months after herbicide treatment slide recovery times.

Colonization of slides by Hantzschia and Oscillatoria is shown in Fig. 1. Data represent the numbers of cells of each organism observed on the part of the slides corresponding to the top 1 cm of the soil, and are the means of values for 6 replicate slides at each treatment level. Treatment levels at which colonization was significantly different from that on control slides (slides from plots receiving no trifluralin and no simulated acid rain) are shown in Table 1.

Concerning data for Hantzschia in unacidified soil, the trifluralin appeared to have reduced colonization of slides recovered both 3 and 12 months after herbicide treatment (Fig. 1). However, the apparent reduction was significant only on slides recovered after 12 months (Table 1). In plots sprayed with simulated acid rain, significant reductions were observed at 3 and 12 months (pH 4.5) and at 3 months (pH 3.5) after trifluralin application (Table 1). In most cases, colonization of slides removed from soils which were acidified but not treated with herbicide was not significantly different from colonization of control slides (Table 1).

For Oscillatoria in unacidified soil, some stimulation of colonization was apparent for slides recovered from plots 3 months after treatment with 1 mg/kg trifluralin (Fig. 1). However, this was not significant (Table 1). There was likewise no significant difference between colonization at 1 mg/kg trifluralin and that in control plots after 12 months. For slides recovered from unacidified plots treated with 2 mg/kg trifluralin, a significant decrease in colonization over that on control slides was observed at both 3 and 12 months after

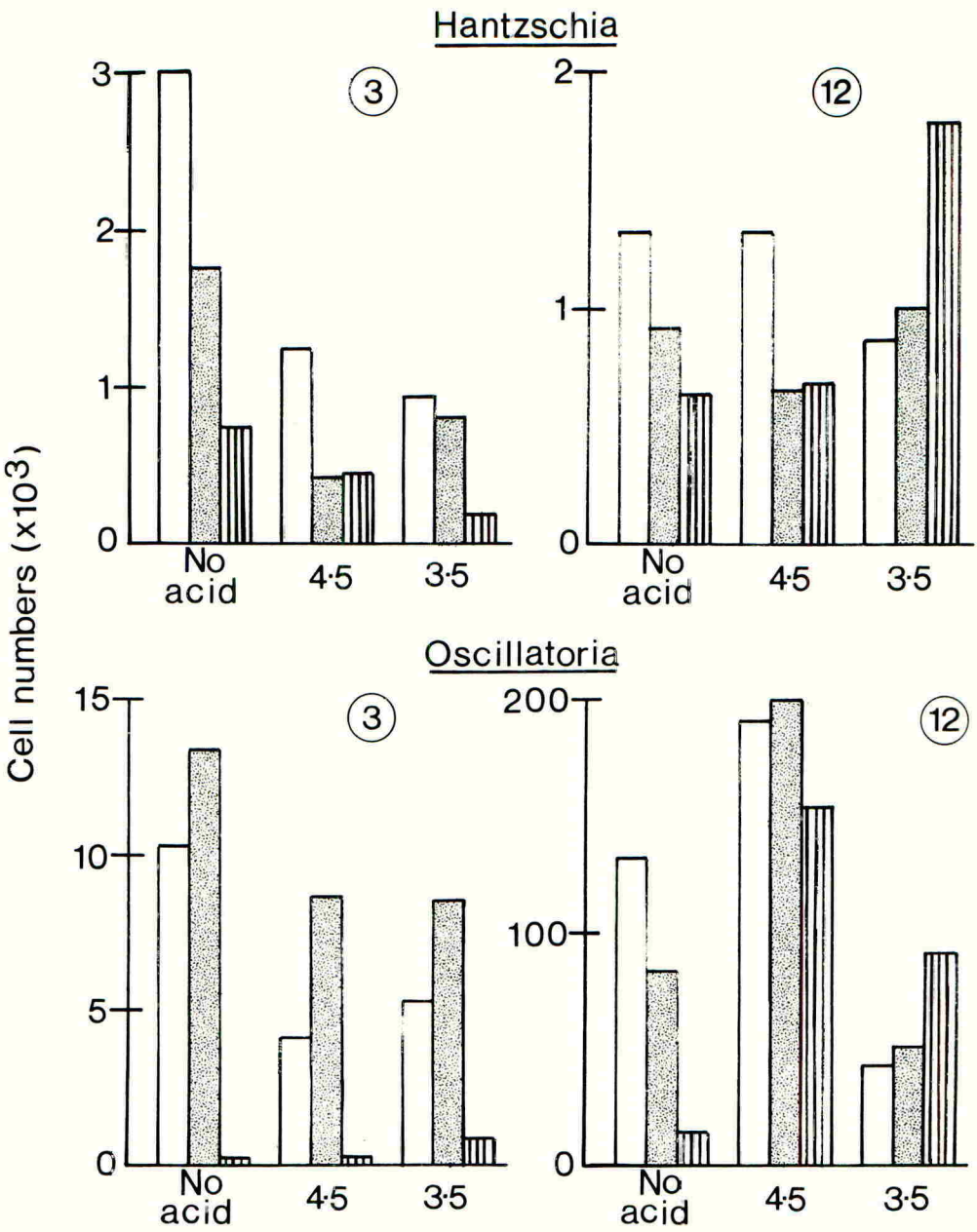


Fig. 1. Number of cells of Hantzschia and Oscillatoria counted on slides recovered from plots 3 and 12 months after treatment with trifluralin (months indicated inside circles). Bars represent data for trifluralin concentrations as follows: □ 0 mg/kg; ▨ 1 mg/kg; ▥ 2 mg/kg. The numbers 4.5 and 3.5 indicate the pH of the simulated acid rain treatments.

TABLE 1

Statistical analysis of colonization at each treatment level as compared with that on control[a] slides

Treatment level		Colonizing organism			
Trifluralin (mg/kg)	Simulated acid rain (pH)	Hantzschia		Oscillatoria	
		3	12	3	12
1	None	NSD	*	NSD	NSD
2	None	NSD	**	*	*
0	4.5	NSD	NSD	NSD	NSD
1	4.5	*	**	NSD	NSD
2	4.5	*	*	*	NSD
0	3.5	NSD	*	NSD	NSD
1	3.5	*	NSD	NSD	NSD
2	3.5	*	NSD	*	NSD

[a]No trifluralin, no simulated acid rain.
The numbers 3 and 12 refer to months after treatment with trifluralin.
NSD - No significant difference.
* - Significantly different from control data at 0.05 level.
** - Significantly different from control data at 0.01 level.

herbicide application (Table 1). In plots sprayed with simulated acid rain, the only treatments which produced data significantly reduced from that for control slides were both of the 2 mg/kg trifluralin treatments after 3 months (Table 1).

DISCUSSION

It is assumed in the following discussion that colonization of implanted slides is indicative of growth of the colonizing organisms in the soil. Accordingly, it is evident that while the growth of the diatom Hantzschia was unaffected 3 months after treatment with trifluralin in unacidified soil, inhibition had occurred by 12 months after treatment. This indicates a somewhat delayed response to the herbicide treatment. It is generally believed that the persistence of trifluralin in soils is 6 months or less (Anderson, 1977), depending, of course, on soil conditions. The herbicide is known to be degraded by a variety of different mechanisms and pathways (Probst et al., 1975). It is possible that in the present study, degradation products forming between the 3 and 12 month sampling times may have been more harmful to Hantzschia than

was the parent compound. In support of this statement, the dealkylation known to occur in the degradation of trifluralin can result in an increase in biological activity (Hill, 1978). Metabolites resulting from dealkylation are, however, thought to be short-lived in the soil (Cripps & Roberts, 1978).

In soils sprayed with simulated acid rain following trifluralin treatment, inhibition of Hantzschia was more pronounced than that noted above, and significant reduction of growth was observed 3 months after herbicide treatment. Growth of the diatom in plots treated with acid alone (no trifluralin) was unaffected. It therefore appears that while the acid at the pH levels and application rates employed was in itself harmless to Hantzschia, it may have enhanced the susceptibility of the alga to trifluralin in the soil. This was observed less clearly 12 months after herbicide treatment. In order to interpret these data, it is necessary to consider how the acid might affect firstly the mode of action of trifluralin on Hantzschia cells, and secondly the behaviour of the herbicide in the soil.

Addressing the first of these points, sulphuric acid is a contact herbicide, weakening and disorganizing cellular membranes (Anderson, 1977). Trifluralin is a mitotic inhibitor (Probst et al., 1975) and can adversely affect development of cell membranes and cell walls during mitosis (Anderson, 1977). The deleterious effect of herbicide-induced impairment of development of cell membranes and walls may therefore have been enhanced by the contact effect of the acid, hastening the weakening and disorganization of these structures.

Concerning the possible effect of the acid on the behaviour of trifluralin in the soil, this may have included the following phenomena. The acid may have affected herbicide degradation, accelerating the formation of metabolites which might be more toxic than the parent compound to Hantzschia. Soil acidity has been observed to enhance photodecomposition of trifluralin by nitro group reduction (Probst et al., 1975), but according to Hill (1978), the loss of the nitro group usually results in loss, not increase, of biological activity. The acid may also have affected the extent of adsorption of the trifluralin to the soil. Trifluralin is known to become quite strongly adsorbed to soil, the extent of which is affected by a variety of factors including pH (Anderson, 1977). It is possible that the acid treatments reduced the extent of adsorption of the trifluralin, rendering it more available for uptake by, and more harmful to, the Hantzschia cells.

In concluding the discussion of the apparent acid-enhanced action of trifluralin on Hantzschia, it should be mentioned that long-term alteration in soil pH was not observed at any time during the study. Measurements made each week immediately before acid treatment, and at the conclusion of the experiment, showed soil pH in all plots to be at its normal level.

The growth of Oscillatoria in the soil was less affected by the treatments than was that of Hantzschia; under no circumstances was trifluralin at 1 mg/kg observed to affect the Cyano-

bacterium. This apparent greater resistance of Oscillatoria to the herbicide and the acid may have a morphological explanation. The Oscillatoria was present on the slides in massive clumps of trichomes, rather than as scattered cells as was the case for Hantzschia. It is reasonable to assume similar growth habits of the organisms in the soil itself. The cells in the centre of large clumps of Oscillatoria may therefore be physically protected against chemicals such as trifluralin and sulphuric acid applied to the soil. This protection may be further enhanced by the presence of mucilaginous sheaths covering the Oscillatoria filaments.

Throughout this discussion, the assumption has been made that colonization of slides reflects the growth of colonizing organisms in the soil itself, implying that any reduction of colonization is indicative of mortality of the organisms. The possibility that mortality may not necessarily be indicated should be entertained. The treatments used in the study may instead have affected the ability of the organisms to colonize the glass slides. For example, motility may have been impaired, preventing the organisms from moving towards the slides prior to colonization. Such possible sub-lethal physiological effects are as much a cause for attention as are direct lethal effects (Pipe & Cullimore, 1984).

It is evident from the data presented in this paper that, at field application rates, trifluralin can exert a significant effect on members of the soil algal and cyanobacterial flora for up to 12 months after application. Should acid rain fall on soils containing trifluralin, the effect on these organisms can be expected to be even more pronounced. Such an impairment of the normal growth of algae and Cyanobacteria may seriously impede their participation in the maintenance of soil fertility.

ACKNOWLEDGEMENTS

The technical assistance of Mr. G.B. Moore is gratefully acknowledged.

REFERENCES

Anderson, W.P. (1977) Weed Science: Principles West Publishing Company.

Cripps, R.E.; Roberts, T.R. (1978) Microbial degradation of herbicides. In: Pesticide Microbiology I.R. Hill and S.J.L. Wright (Eds), Academic Press Inc. (London) Ltd., pp. 669-730.

Fogg, G.E.; Stewart, W.D.P.; Fay, P.; Walsby, A.E. (1973) The Blue-Green Algae New York: Academic Press.

Hill, I.R. (1978) Microbial transformation of pesticides. In: Pesticide Microbiology I.R. Hill and S.J.L. Wright (Eds), Academic Press Inc. (London) Ltd., pp. 137-202.

McCann, A.E.; Cullimore, D.R. (1979) Influence of pesticides on the soil algal flora. Residue Reviews 72, 1-31.

Pipe, A.E.; Cullimore, D.R. (1980) An implanted slide technique for examining the effects of the herbicide diuron on soil algae. Bulletin of Environmental Contamination and Toxicology 24, 306-312.

Pipe, A.E.; Cullimore, D.R. (1984) Influence of five phenylurea herbicides on the diatom Hantzschia in a sandy loam soil. Bulletin of Environmental Contamination and Toxicology 33, 439-443.

Pipe, A.E.; Shubert, L.E. (1984) The use of algae as indicators of soil fertility. In: Algae as Ecological Indicators L.E. Shubert (Ed), Academic Press, pp. 213-233.

Pramer, D.; Schmidt, E.L. (1964) Experimental Soil Microbiology Burgess Publishing Company, Minneapolis.

Probst, G.W.; Golab, T.; Wright, W.L. (1975) Dinitroanilines. In: Herbicides: Chemistry, Degradation and Mode of Action P.C. Kearney and D.D. Kaufman (Eds), Second edition, Marcel Dekker Inc., pp. 453-500.

Snedecor, G.W.; Cochran, W.G. (1967) Statistical Methods Iowa State University Press, Ames.

ACCELERATED BIODEGRADATION OF PESTICIDES IN SOIL AND ITS EFFECT ON PESTICIDE EFFICACY

D. D. KAUFMAN

Soil-Microbial Systems Laboratory, BARC-East, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, United States

ABSTRACT

The biodegradation processes associated with a given pesticide may be influenced by the rate and frequency of its application, the cropping system, and the presence of other pesticides applied either simultaneously or sequentially, in addition to the chemical and physical characteristics of both the soil and the chemical itself, and the prevailing environmental conditions. The interactions of these factors may result in either accelerated or reduced rates of biodegradation thereby affecting efficacy. Frequent, repeated applications of many pesticides are known to result in accelerated rates of biodegradation of these pesticides in soils. Some pesticides may act as inducers of microbial enzymes which degrade other pesticides, even though they themselves are not necessarily substrates for the microbial enzymes induced. Other pesticides may inhibit the biodegradation of some pesticides in soil. Recognition and an understanding of these interactions is important if we are to preserve these chemicals as important agricultural tools.

INTRODUCTION

The accelerated biodegradation of some pesticides in soil and the accompanying loss of pest control is becoming an increasingly important problem for the farmer, the agricultural scientist, consultant and advisor, and the agrichemical industry. With the development of synthetic fertilizers and pesticides, it became feasible to grow many crops continuously rather than in rotation with one another. The production of fewer crops continuously offered a sufficiently large economic advantage that many farmers readily adopted the associated pest control strategies and began using the same chemicals in the same fields almost habitually. By early to mid 1970's extension agents began receiving performance complaints about several of the pesticides being used more regularly. Although it may be common to have some performance complaints with most heavily used pesticides, complaints became more consistent with some products (Tollefson 1986; Rahman et al. 1979). In the U.S. many of these initial problems evolved around the use of methylcarbamate insecticides and thiocarbamate herbicides in continuous corn.

The development of microbial populations capable of rapidly degrading sequential applications of pesticides had been demonstrated under laboratory conditions for several pesticides: phenoxyalkanoates (Audus, 1951); chlorpropham (Kaufman & Kearney 1965); dalapon (Kaufman 1964); and several phenylamides (Kaufman & Blake 1973). The occurrence of this phenomenon under field conditions has also been described. Hurle and Rademacher (1970) compared the dissipation of DNOC and 2,4-D in soil treated for the first time and soil from field plots treated annually over a period of 12 years. 2,4-D dissipation was more rapid in previously treated soil than in soil treated for the first time, whereas pretreatment had no effect on the

rate of EPTC dissipation from soil. Similar promotions have been obtained with 2,4-D (Kirkland & Fryer 1966, 1972; Newman & Thomas 1949; Aly & Faust, 1964; Fryer & Kirkland 1970), endothall (Horowitz 1966), but not with simazine or linuron (Fryer & Kirkland 1970). The time for MCPA applications to reach the limit of detection was reduced from 3 weeks after three previous applications to 4 days after 10 previous applications.

The actual significance of this phenomenon in soil would be of minor consequence to pesticides which are primarily active as foliar or aerial contact chemicals. Pesticides which are primarily active in soil or through root absorption from soil, however, could expect to have limited effectiveness. Research efforts in my laboratory have been directed at examining the degradation of thiocarbamate, methylcarbamate, phenylamide and organophosphate pesticides in their respective problem and nonproblem soils, and to ascertain what effect, if any, these problem soils may have on the persistence and performance of structurally related pesticides. "Problem" and "nonproblem" soils used in these investigations are defined as: (a) Problem soil, soil in which the chemical applied failed to control the target pest; and (b) Nonproblem soil, an identical soil type with an identical cropping history, but without any known use of any chemical, or an identical soil type from an untreated border area (fence row) adjacent to a problem field.

MATERIALS AND METHODS

We examined the degradation of ^{14}C-ethyl-and ^{14}C-propyl-EPTC in three pairs of problem-nonproblem soils, the degradation of ^{14}C-carbonyl carbofuran in five pairs of problem-nonproblem soils, and the degradation of ^{14}C-ethyl- and ^{14}C-methylthio terbufos in five pairs of problem-nonproblem soils. All soils were obtained from our Midwestern corn producing areas where most of these problems have appeared. All soils were received in a fresh moist condition from field locations, and were immediately sieved through a 2 mm No. 10 U.S. Standard sieve prior to storage in polyethylene bags at 5°C. The persistence and degradation of the appropriate ^{14}C-labeled pesticide and structurally related ^{14}C-herbicides, insecticides, and fungicides were then examined in each soil. All soil metabolism experiments were performed by incubating ^{14}C-pesticide treated soils in soil biometer flasks. At periodic intervals treated soils were sacrificed, extracted and analyzed for ^{14}C content and product distribution.

RESULTS

The results of investigations with ^{14}C-ethyl-EPTC in EPTC problem and nonproblem soils are shown in Figure 1. Degradation of EPTC with evolution of $^{14}CO_2$ from the ethyl moiety occurred far more rapidly in EPTC problem soils than in nonproblem soils. Soil sterilization by either autoclaving or gamma irradiation drastically reduces the rate of EPTC degradation. Similar results have been described by others with EPTC (Obrigawitch et al. 1982a,b, 1983; Wilson 1984), butylate (Skipper et al. 1986; Obrigawitch et al. 1983; Wilson 1984), and vernolate (Wilson 1984). Degradation of carbofuran with evolution of $^{14}CO_2$ from the carbonyl position occurred far more rapidly in carbofuran problem soils than in nonproblem soils (Figure 2). Felsot et al. (1981), Harris et al. (1984), and Read (1986) also demonstrated an accelerated degradation of carbofuran in soils having a history of carbofuran use. The inhibition of degradation of these chemicals by antibiotics added to the soil, or by soil

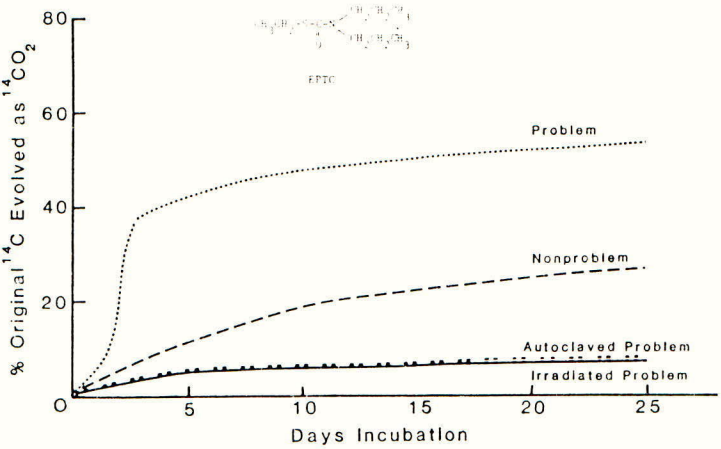


Fig. 1. Degradation of ^{14}C-ethyl-EPTC in Eradicane problem (sterile and nonsterile) and nonproblem soils.

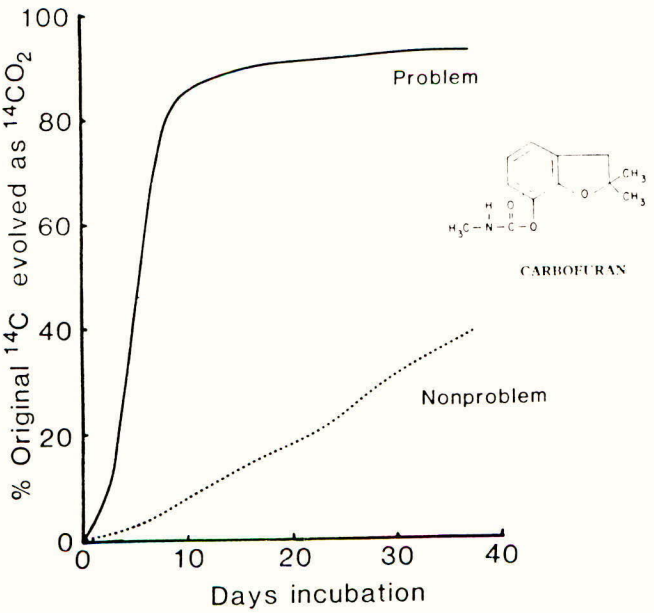


Fig. 2. The degradation of ^{14}C-carbonyl-carbofuran in carbofuran problem and nonproblem soils.

sterilization (autoclaving or gamma irradiation) confirmed the importance of an active microbial population in the degradation of these chemicals.

Soil microorganisms capable of metabolizing either EPTC or carbofuran have been isolated from problem soils and characterized. Tam et al. (1987) described the isolation of an Arthrobacter strain which metabolized EPTC as a sole source of carbon. Venkateswarlu and Sethunathan (1985) isolated Pseudomonas cepacia and a Nocardia sp. from flooded alluvial soil amended with carbofuran which metabolized carbofuran fairly rapidly in mineral salts medium or soil extract agar supplemented with yeast extract. Karns et al. (1986) isolated an Achromobacter species capable of rapidly utilizing carbofuran as a sole source of nitrogen. Degradation of EPTC was mediated by a 50.5 MDA plasmid in the Arthrobacter cells (Tam et al. 1987). The loss of this plasmid resulted irreversibly in mutants unable to degrade EPTC. Whether or not the carbofuran degradation capacity of Achromobacter is associated with a plasmid chromosome has not yet been determined (Karns et al. 1986). The role of plasmids in microbial degradation of certain organophosphates (Serdar et al. 1982) and chlorinated compounds such as benzoates and phenoxyalkanoates is also known (Tomasek et al. 1986). It has been suggested (Waid 1972) that the ability of adapted populations to persist for many months in the presumed absence of the substrate must result from the conservation of a very effective genetic mechanism within the soil microflora even though it has no apparent survival value among individual cultures in the absence of an appropriate substrate. Several review articles on degradative plasmids and their molecular nature and mode of evolution are available (Farrell & Chakrabarty 1979; Chakrabarty 1976).

The correlation of laboratory results as shown here to field performance and efficacy problems of thiocarbamate herbicides and carbofuran insecticide has been demonstrated by numerous investigators (Skipper et al. 1986; Obrigawitch et al. 1982a,b, 1983; Wilson 1984; Lee et al. 1984; Kaufman & Edwards 1983; Kaufman et al. 1985; Nelsot et al. 1981; Read 1986) and others. Similar observations have been also described with other pesticides: the insecticides aldicarb (Read, 1987), parathion (Sethunathan 1973), and diazinon (Sethunathan & Pathak 1971); the fungicides DCNA (Groves & Chough 1970), benomyl and carbendazim (Yarden et al. 1985), and iprodione (Entwistle 1983); and the herbicides 2,4-D, dalapon, chlorpropham, propham, TCA, pronamide, napropamide, bensulide, alachlor and diethatyl (Gray & Joo 1985), endothall (Horowitz 1966), amitrole (Riepma 1962) and diphenamid (Katan et al. 1984), and the nematicide ethoprop (Rohde et al. 1980).

DISCUSSION

There are several aspects of the phenomenon of accelerated biodegradation which should be given serious consideration. First of all, the phenomenon is a natural process which, in fact, was first described in relationship to pesticides nearly four decades ago by Audus (1951) in his research on the biodegradation of the herbicide 2,4-D. His work, and the work of many subsequent investigators clearly demonstrated that once a microbial population adapted to degradation of a foreign molecule, in this case a pesticide, subsequent applications would be degraded much more rapidly. Much of the initial lag period involves the adaptation process and the development of a population level, or metabolic rate sufficient to detect a significant loss of the pesticide. Once the process has been established subsequent applications will be degraded without the lag period

which occurred with the first application. A more surprising aspect of this phenomenon is the persistence of the effect from one growing season to another. While plant pathogenic organisms are known to survive for several years on plant debris or alternate hosts, the persistence of a pesticide degrading population much beyond one year was not considered likely. Results of numerous investigators (Fryer & Kirkland 1970; Kirkland & Fryer 1972; Newman et al. 1952; Torstensson et al. 1975) have revealed otherwise, however. As new information is developed on the existence and persistence of pesticide degrading plasmids, we will gain new insights into the persistence of these important biological factors in soil.

A second important part in the characterization of the accelerated degradation of pesticides involves the presence of adequate pest pressure. In some of our early investigations we compared the degradation of selected insecticides in soils from fields where a consecutive use pattern had been established but no efficacy problems were being observed, with their degradation in soils from fields where efficacy problems had been observed. The same rapid rate of degradation was observed in both soil types. Further investigation ultimately led us to the understanding that in the absence of adequate pest pressure a farmer may not recognize that the chemical applied is no longer able to be as effective as it was initially.

A third important consideration in the development of a problem soil for an individual chemical is the effect of that microbial population on the biodegradation and persistence of other structurally related chemicals used thereafter. Ample information exists which clearly demonstrates that isolated microorganisms capable of degrading one pesticide are frequently capable of degrading other similar pesticides either more or less efficiently (Audus 1960; Engelhardt et al. 1971, 1973; Blake & Kaufman 1975; Kaufman & Blake 1973; Kaufman 1977; Kaufman et al. 1985). The mechanisms whereby this occurs are discussed in more detail elsewhere (Kaufman et al. 1985; Roeth 1986) The implications of such observations are clear and must be considered when attempts are made to reestablish pest control in soils where accelerated biodegradation of one or more chemicals has been observed. To date, cross degradation or cross enhancement has been detected in the field within specific chemical classes, i.e., thiocarbamates (Roeth 1986), methylcarbamates (Harris et al. 1984), etc. Read (1983), however, reported a cross enhancement occurred between the methylcarbamate insecticide carbofuran and the organophosphate insecticide fensulfothion. Other cross enhancements have been observed in the laboratory with soils taken from problem field sites (Kaufman et al. 1985).

Developing suitable methods for (a) regulating the rate of pesticide biodegradation in soil, (b) preventing the development of problem soils, and (c) controlling or eradicating microbial populations in problem soils once they have developed is essential for maintenance of adequate pest control with soil-applied chemicals. At present essentially no information is available on how, once a problem field has developed, it can be converted back to a nonproblem field for the chemical involved. Although soil sterilization might be effective in high-cash-return crops, it would not be economically feasible for most field crop soils. Thus, new technology is needed on how to reclaim problem fields. Rotation of chemicals has shown some degree of success in preventing problem field development, but the long range effectiveness of this preventive measure is not known. Crop rotation has been tentatively examined but is only

successful when the pesticide complex used on one crop is suitably different from that used on the rotational crop, or the rotation sufficiently interferes with the life cycle of the pest. Several microbe or enzyme inhibitors appear effective for extending soil persistence (Kaufman et al 1985; Obrigawitch et al. 1982a; Roeth 1986), but the technology involved needs further development.

The practical significance of these observations is of considerable importance to agriculture. Successful pest control depends upon maximum performance of our pest control chemicals. The deliberate combination of certain pesticides, or addition of microbial or enzyme inhibitors to pesticide formulations, or the rotation of certain crops and pesticides shows considerable promise for the purpose of controlled persistence of biodegradable pesticides.

REFERENCES

Aly, O.M.; Faust, S.D. (1964) Studies on the fate of 2,4-D and ester derivatives in natural surface water. Journal Agricultural and Food Chemistry 12, 541-546.

Audus, L.J. (1951) The biological detoxification of hormone herbicides in soil. Plant Soil 3, 170-192.

Audus, L.J. (1960) Microbiological breakdown of herbicides in soils. In: Herbicides and the Soil E.K. Woodford and G.R. Sagar (eds), Oxford: Blackwell Scientific Publishers, pp. 1-16.

Blake, J; Kaufman, D.D. (1975) Characterization of acylanilide-hydrolyzing enzyme(s) from Fusarium oxysporum schlecht. Pesticide Biochemistry and Physiology 5, 305-313.

Chakrabarty, A.M. (1976) Plasmides in Pseudomonas. Annual Review of Genetics 10, 7-30.

Engelhardt, G.; Wallnofer, P.R.; Plapp, R. (1971) Degradation of linuron and some other herbicides and fungicides by a linuron-inducible enzyme obtained from Bacillus sphaericus. Applied Microbiology 22, 284-288.

Engelhardt, G.; Wallnofer, P.R.; Plapp, R. (1973) Purification and properties of an aryl acylamidase of Bacillus sphaericus catalyzing the hydrolysis of various phenylamide herbicides and fungicides. Applied Microbiology 26, 709-718.

Entwistle, A. R. (1983) Changes in the effectiveness of iprodione for the control of allium white rot. Phytopathology 73, 800.

Farrell, R.; Chakrabarty, A.M. (1979) Degradative plasmids: Molecular nature and mode of evolution. In: Plasmids of Medical, Environmental and Commercial Importance K.N. Timmis and A. Puhler (eds), Elsevier/North-Holland: Biomedical Press, pp. 97-109.

Felsot, A.; Maddox, J.V.; Bruce, W. (1981) Enhanced microbial degradation of carbofuran in soils with histories of Furadan use. Bulletin Environmental Contamination and Toxicology 24, 778-782.

Fryer, J.D.; Kirkland, K. (1970) Field experiments to investigate long-term effects of repeated applications of MCPA, tri-allate, simazine and linuron: Report after 6 years. Weed Research 10, 133-158.

Groves, K.; Chough, K.S. (1970).Fate of the fungicide, 2,6-dichloro-4-nitroaniline (DCNA) in plants and soils. Journal Agricultural and Food Chemistry 18, 1127-1128.

Gray, R.A.; Joo, C.K. (1985) Reduction in weed control after repeated applications of thiocarbamate and other herbicides. Weed Science 33, 698-702.

Harris, C.R.; Chapman, R.A.; Harris, C.; Tu, C.M. (1984) Biodegradation of pesticides in soil: Rapid induction of carbamate degrading factors

after carbofuran treatment. Journal Environmental Science Health Sect. B. 19, 1-11.
Horowitz, M. (1966) Breakdown of endothal in soil. Weed Research 6, 168-171.
Hurle, D.; Rademacher, B. (1970) The effect of long-term treatment with DNOC and 2,4-D on their breakdown in soil. Weed Research 10, 159-164.
Karns, J.S.; Mulbry, W.W.; Kearney, P.C. (1986) Biotechnology in pesticides environmental research. In: Biotechnology for Solving Agricultural Problems: BARC Symposium 10 P.C. Augustine, H.D. Danforth, M.R. Bakst (eds), Boston: Martinus Nijhoff Publishers, pp. 339-354.
Katan, J.; Wilson, H.P.; Kaufman, D.D. (1984) Enhanced degradation of diphenamid and other pesticides in herbicide history soils. Abstracts Weed Science Society of America, No. 253.
Kaufman, D.D. (1964) Microbial degradation of 2,2-dichloropropionic acid in five soils. Canadian Journal of Microbiology 10, 843-852.
Kaufman, D.D. (1977) Biodegradation and persistence of several acetamide acylanilide, azide, carbamate, and organophosphate pesticide combinations. Soil Biology and Biochemistry 9, 49-57.
Kaufman, D.D.; Blake, J. (1973) Microbial degradation of several acetamide, acylanilide, carbamate, toluidine and urea pesticids. Soil Biology and Biochemistry 5, 297-308.
Kaufman, D.D.; Edwards, D.F. (1983) Pesticide/Microbe interaction effects on persistence of pesticides in soil. In: IUPAC Pesticide Chemistry: Human Welfare and the Environment J. Miyamoto et al. (eds), N.Y.: Pergamon Press, pp. 177-182.
Kaufman, D.D.; Katan, Y.; Edwards, D.F.; Jordan, E.G. (1985) Microbial adaptation and metabolism of pesticides. In: Agricultural Chemicals of the Future: BARC Symposium 8 J.L. Hilton (ed), Totowa, N.J.: Rowman and Allanheld, pp. 437-451.
Kaufman, D.D.; Kearney, P.C. (1965) Microbial degradation of isopropyl-N-3-chlorophenylcarbamate and 2-chloroethyl-N-3-chlorophenylcarbamate. Applied Microbiology 13, 443-446.
Kirkland, K.; Fryer, J.D. (1966) Pretreatment of soil with MCPA as a factor affecting persistence of a subsequent application. Proc. 10th British Weed Control Conference, p. 616.
Kirkland, K.; Fryer, J.D. (1972) Degradation of several herbicides in soil previously treated with MCPA. Weed Research 12, 90-95.
Lee, A.; Rahman, A.; Holland, P.T. (1984) Decomposition of the herbicide EPTC in soils with a history of previous EPTC applications. New Zealand Journal of Agricultural Research 27, 201-206.
Newman, A.S.; Thomas, J.R. (1949) Decomposition of 2,4-dichlorophenoxy-acetic acid in soil and liquid media. Proceedings Soil Science Society of America 14, 160-164.
Newman, A.S.; Thomas, J.R.; Walker, R.L. (1952) Disappearance of 2,4-dichlorophenoxyacetic acid from soil. Proceedings Soil Science Society of America 6, 21-24.
Obrigawitch, T.; Martin, A.R.; Roeth, F.R. (1983) Degradation of thiocarbamate herbicides in soils exhibiting rapid EPTC breakdown. Weed Science 31, 187-192.
Obrigawitch, T.; Roeth, F.W.; Martin, A.R.; Wilson, Jr., R.G. (1982a) Addition of R-33865 to EPTC for extended herbicide activity. Weed Science 30, 417-422.
Obrigawitch, T.; Wilson, R.G.; Martin, A.R.; Roeth, F.W. (1982b) The influence of temperature, moisture, and prior EPTC application on the degradation of EPTC in soils. Weed Science 30, 175-181.
Rahman, A.; Atkinson, G.C.; Douglas, J.A.; Sinclair, D.P. (1979) Eradicane

causes problems. New Zealand Journal Agriculture 139, 47-49.
Read, D.C. (1983) Enhanced microbial degradation of carbofuran and fensulfothion after repeated applications to acid mineral soil. Agriculture, Ecosystems and Environment 10, 37-46.
Read, D.C. (1986) Accelerated microbial breakdown of carbofuran in soil from previously treated fields. Agriculture, Ecosystems and Environment 15, 51-61.
Read, D.C. (1987) Greatly accelerated microbial degradation of aldicarb in re-treated field soil, in flooded soil, and in water. Journal of Economic Entomology 80, 156-163.
Riepma, P. (1962) Preliminary observations on the breakdown of 3-amino-1,2,4-triazole in soils. Weed Research 2, 41-50.
Roeth, F.W. (1986) Enhanced herbicide degradation in soil with repeat application. Reviews of Weed Science 2, 45-167.
Rohde, W.A.; Johnson, A.W.; Bowler, C.C.; Glaze, N.C. (1980) Influence of climate and cropping patterns on the efficacy of ethoprop, methyl bromide and DD-MENCS for control of rootknot nematodes. Journal of Nematology 12, 33-39.
Serdar, C.M.; Gibson, D.T.; Munnecke, D.E.; Lancaster, J.H. (1982) Plasmid involvement in parathion hydrolysis by Pseudomonas diminuta. Applied Environmental Microbiology 44, 246-249.
Sethunathan, N. (1973) Degradation of parathion in flooded acid soils. Journal of Agriculture and Food Chemistry 21, 602-604.
Sethunathan, N.; Pathak, M.D. (1972) Increased biological hydrolysis of diazinon after repeated application in rice paddies. Journal of Agricultural and Food Chemistry 20, 586-589.
Skipper, H.D.; Murdock, E.C.; Gooden, D.T.; Zublena, J.P.; Amakiri, M.A. (1986) Enhanced herbicide biodegradation in South Carolina soils previously treated with butylate. Weed Science 34, 558-563.
Tam, A.C.; Behki, R.M.; Khan, S.U. (1987) Isolation and characterization of an s-ethyl-N,N-dipropylthiocarbamate-degrading Arthrobacter strain and evidence for plasmid-associated s-ethyl-N,N-dipropylthiocarbamate degradation. Applied and Environmental Microbiology 53, 1088-1093.
Tollefson, J. (1986) Accelerated degradation of soil insecticides used for corn rootworm control. Proceedings 1986 British Crop Protection Conference 8C-25, 1131-1136.
Tomasek, P.H.; Frantz, B.; Chatterjee, D.K.; Chaknabarty, A.M. (1986) Genetic and molecular basis of the microbial degradation of herbicides and pesticides. In: Biotechnology for Solving Agricultural Problems: BARC Symposium 10 P.C. Augustine, H.D. Danforth, M.R. Bakst (eds), Boston: Martinus Nijhoff Publishers, pp. 355-383.
Torstensson, T.L.; Stark, J.; Goransson, H. (1975) The effect of repeated applications of 2,4-D and MCPA on their breakdown in soil. Weed Research 15, 159-164.
Venkateswarlu, K.; Sethunathan, N. (1985) Enhanced degradation of carbofuran by Pseudomonas cepacia and Nocardia sp. in the presence of growth factors. Plant and Soil 84, 445-449.
Waid, J.S. (1972) The possible importance of transfer factors in the bacterial degradation of herbicides in natural ecosystems. Residue Reviews 44, 65-71.
Wilson, R.G. (1984) Accelerated degradation of thiocarbamate herbicides in soil with prior thiocarbamate exposure. Weed Science 32, 264-266.
Yarden, O.; Ben-Yephet, Y.; Katan, J.; Aharonson, N. (1986) Fungicidal control of Sclerotinia sclerotiorum in soil with a combination of benomyl and thiram. Plant Disease 70, 738-742.

DECOMPOSITION OF EPTC BY SOIL MICROBES IN TWO SOILS

I. NAGY, J. NAGY, J. MATYAS

North-Hungarian Chemical Works, Sajobabony, Hungary

M. KECSKES

University of Agricultural Sciences, Godollo, Hungary

ABSTRACT

The decomposition of the herbicide EPTC by soil microbes was investigated in two soil types with and without previous EPTC and EPTC degrader microbe exposure. We isolated a bacterial strain grown on EPTC as sole carbon and energy source. The loam soil (I) without previous EPTC exposure was divided into subsamples which were I.a. autoclaved, I.b. control, I.c. treated with 6 kg/ha EPTC two times, I.d. autoclaved and inoculated with the EPTC degrader strain (HE2), I.e. inoculated with strain HE2, I.f. inoculated with 10% of 16 years previously EPTC exposed soil (II). The other soil was a brown forest soil with 16 years previous EPTC exposure (II). The decomposition of EPTC was faster in all fresh soil samples than in the autoclaved control. The fastest decompositions were in I.c.d.e. samples (5-7 days), in the II. soil it was 10 days, while in I.b.f. samples it was 14 days. The decomposition of EPTC in I.b. and II. soils was nearly equal. For this reason the decomposition did not accelerate in I.f. samples although expected, while the decomposition of EPTC accelerated in I.c.d.e. samples as expected.

INTRODUCTION

The accelerated decomposition of the herbicide EPTC (s-ethyl N, N-dipropylthiocarbamate) with the antidote R-25788 (N, N-diallyl-2, 2-dichloroacetamide) which was used for controlling grass weeds in maize fields was reported from New Zealand maize fields where EPTC exposure (Rahman et al., 1979). Studying this phenomenon, it was reported that soil microorganisms were the major reason for the lack of weed control (Gray, 1979; Rahman et al., 1981) and it was shown that some, but not all soils could be conditioned by repeated applications of EPTC but that there were many other factors which determined whether a soil would become conditioned, besides repeated application (Capper, 1982).

Several chemicals were tested by Stauffer Chem. Co. to improve the efficacy of weed control obtained with EPTC + R-25788 when applied to conditioned and normal soils. One chemical, R-33865 (O, O-diethyl-O-phenyl-phosphorothioate) was particularly effective in restoring the herbicidal activity of EPTC (i.e. acting as a herbicide extender).

The objectives of the work reported in this paper were:

a to isolate microbes which are able to grow on EPTC as sole C and energy source

b to determine the period of EPTC decomposition in soil samples with and without previous EPTC and EPTC degrader bacterial strain exposure

c to determine the effect of R-33865 on EPTC degraders

MATERIALS AND METHODS

Soils

I. Loam soil (Borsodszirak), with no previous EPTC exposure was used for greenhouse experiments. This soil (50 kg) was treated with 6 kg/ha EPTC in May and August 1986.

II. Brown forest soils (Putnok) with 16 years previous EPTC exposure, which was taken in November 1986, and was used as the EPTC degrader donor.

The soils were stored at 4°C in paper bags for 6 months.

For the list of soil samples, see abstract.

EPTC handlings

The samples were passed through a 2 mm sieve, and gas chromatography did not detect any residual EPTC from field application. EPTC was applied at 50 mg/l to two sub-samples (200 g, oven-dry basis) of each soils according to Lee et al. (1984). The moisture of soils was 30%. The 500 ml flasks of samples were plugged with cotton wool and incubated in the dark at 25°C for 16 days. At 2-3 day intervals , 10 g soil samples were removed aseptically to determine EPTC levels using gas chromatography.

Isolation and selection of EPTC degraders

EPTC degraders were isolated from the I.b.c. and II. soils. Diluted samples (10^{-1} -10^{-7}) were made and 0.1 ml of the dilutions was plated onto basal salt agar + 400 mg/l EPTC medium (BSAEM). The BSAM was prepared from: NH_4NO_3, 0.5g; KH_2PO_4, 0.4g; K_2HPO_4, 1.6G. NaCl, 0.1g; $MgSO_4 \cdot 7H_2O$, 0.2g; $CaCl_2 \cdot 2H_2O$, 0.5mg; $CuSO_4 \cdot 5H_2O$, 0.5mg; $FeSO_4 \cdot 7H_2O$, 0.5mg; $ZnCl_2$, 0.5mg; agar 20g; dist. water, 1000 ml. All types of strains were transferred from countable plates to BSAEM tubes after 10-day incubations at 25°C. The strains were purified on nutrient agar plates and were maintained on BSAEM.

One hundred and twenty-one isolates were investigated for their ability to grow on EPTC as their only C and energy source in 100 ml BS+250 mg/l EPTC (BSEM) by liquid shake culture carried out in triplicate. The EPTC was dissolved into the water by shaking aseptically in Stohman flasks. The samples were inoculated with 1 ml of 1-9 x 10^9 i/ml of suspensions of strains grown on nutrient medium.

The growth of strains was controlled photometrically measuring the turbidity of the initial (1-9 x 10^7) and the final state (after 14 days) at 530 nm.

By this method we found only one bacterial strain which was able to grow on EPTC. This strain was isolated from loam I.c. soil (two previous EPTC exposures). this was marked as strain HE2. The identification of HE2 strain is in progress.

Investigation of EPTC degrader strain

The decomposition of EPTC was investigated by pure culture of HE2 strain. The 200 ml of BSEM (in duplicate) was inoculated with 2.3 x 10^7 i/ml HE2, and was completed with 0.1; 1; 10; 100 mg/l of R-33865, when we investigated the effect of this extender. The flasks were shaken two times per day for two mins and were incubated at 25°C for 33 days. Then, 10 ml of samples were anlaysed for EPTC by gas chromatography for 2-3 days.

GLC determination of EPTC

The EPTC in 10 g soil samples was extracted into 25 ml of petroleum ether (70°C BP) acetone reagent (3:2 v/v) according to Lee et al. (1984).

The petroleum ether extract (1.5 µl) was injected into a CHROM-4 gas chromatograph containing a glass column (2.4m x 3mm) packed with Chromosorb W (80/100 mesh) coated with 10% SE-30. The oven temperature was 180°C and the injector temperature was 220°C.

A standard curve of the peak height of EPTC was prepared for EPTC concentrations of 0.25-50 mg/l in petroleum ether. Results were expressed as percentage EPTC recovered from the soil relative to day 0.

RESULTS

Figures 1 and 2 show the fate of EPTC added to soil samples. The loss of EPTC in the autoclaved soil sample was 55% over 15 days because of volatilization.

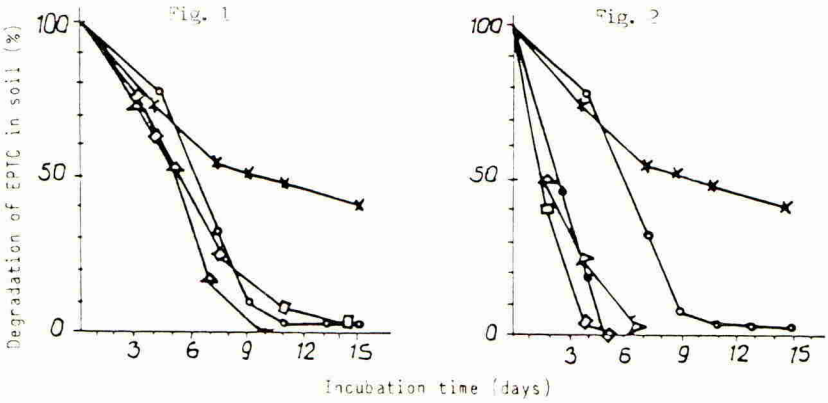


Fig. 1.-2. The degradation of EPTC in soil. X, autoclaved control: O, no previous EPTC exposure; ●, two times previous EPTC exposure; □, autoclaved I.b. soil inoculated with HE2 (2.3x10^7 i/ml); Δ, I.b. soil inoculated with HE2 (2.3x10^7 i/ml); ■, 90% I.b. soil + 10% II. soil; ▲, 16 years previous EPTC exposure (II).

The EPTC was degraded over 14 days in the I.b. (no previous EPTC exposure) and I.f. (I.b. inoculated with 10% of II. soil). The pattern of EPTC loss began as a lag phase of 5 days. This was followed by very rapid decomposition to 9th day and the residual amount of EPTC (4 and 9%) disappeared after 14th day. For the II. soil, the pattern of EPTC decomposition was similar to I.b. and f. samples but was slightly faster and there was not such a long residual decomposition after the 9th day. The EPTC decomposition in I.c.d.e. samples was rapid over 5-7 days. We did not measure the lag phase. There was a two-day difference in the disappearance of EPTC from the I.d. and I.e., which might be explained by the antibiotic sensitivity of HE2 strain.

The HE2 strain was sensitive to: ampicillin, carbenicillin, chloramphenicol, erythromycin, gentamycin, kanamycin, neomycin, penicillin, polymyxin B, streptomycin, tobramycin; and resistent to nalidixic acid.

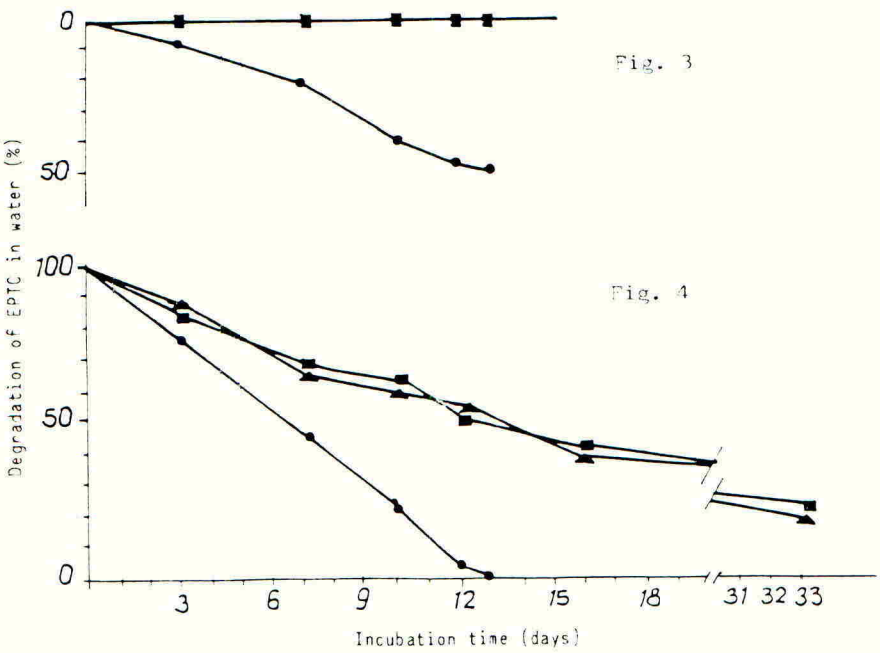


Fig. 3-4. The degradation of EPTC in 250 mg/l EPTC solution without (Fig.3) and with (Fig.4) the EPTC volatilization. ■ , sterile control BSEM; ● , BSEM inoculated with HE2 (2.9×10^7 i/ml); ▲ , BSEM + HE2 + 0.1 mg/l R-33865.

Figures 3 and 4 show the pattern of decomposition of EPTC by HE2 (2.3×10^7 i/ml). The loss of EPTC in sterile control was 50% over 15 days, and 88% over 33 days because of volatilization. In the HE2 inoculated samples the decomposition pattern show a lag phase of 5-6 days, and it is followed by a rapid decomposition to the 13th day when

the EPTC disappeared fron the solution. We measured total inhibitory effect of R-33865 at 0.1; 1; 10 and 100 mg/ concentration on the HE2 strain, and the results of these extender tests were similar to the sterile control.

DISCUSSION

The data obtained during the above described investigation corroborate those reports which showed that the accelerated decomposition of EPTC in soils with previous EPTC exposure was caused by microbes and the activity of these microbes on EPTC inhibited using chemical R-33876. Our results were affected by EPTC volitilization. The losses of EPTC by volitilization were 55% from the soil and 50% from the BSEM over 14 days.

The decomposition of EPTC was faster in all soil samples than in sterile control. In the samples of I.b. (no previous EPTC exposure), I.f. (90% I.b. + 10% II.) and II. (16 years previous EPTC exposure), the decomposition of EPTC was more or less similar, although in the II. soil, it was complete over 10 days. In the case of I.f. soil, faster decomposition was expected by adding 10% of II. soil as a starter microbial inoculum of EPTC degraders, but faster decomposition did not occur because the decomposition of EPTC in the II. soil was unexpectedly similar to the I.b. sample. In the case of soil previously exposed to two EPTC applications (I.c.) and the HE2 inoculated soil samples (I.d.e.), the EPTC decomposition accelerated and the EPTC disappeared over 5-7 days from these soils, without a demonstrable lag phase. For the I.e. sample, the decomposition was slower than I.d. which accounted for the sensitivity of the HE2 strain to antibiotics, and why the HE2 strain was inhibited alightly by the other soil microbes.

The sensitivity of HE2 strain to R-33865 was investigated in BSEM. The growth of HE2 in the control (without R-33865) was good and the pattern of EPTC loss began as a lag phase of 5-6 days. After the 6th day the decomposition accelerated up to the 13th day, when the EPTC disappeared from the medium. The decomposition time and pattern of EPTC in BSEM was similar to the I.b. soil sample.

The activity of the HE2 strain in soil I.d.e. and in BSEM on EPTC was different which was affected by the different EPTC concentration (50 mg/kg and 250 mg/l) and the presence of the other carbon and energy sources in the soil which made the activity of HE2 easier on EPTC according to Lee (1984) who showed that many microbial strains were able to metabolise the 14C EPTC in the presence of glucose.

The chemical R-33865 inhibited the growth of the strain HE2 at 0.1; 1; 10 and 100 mg.1 concentrations when BSEM was with it. The amount of EPTC decreased by volitilization and the patterns of EPTC losses were similar to the sterile control. These results were affected by the high EPTC concentration and the lack of other carbon sources from the BSEM. The presence of other carbon sources decreased the inhibitory effect of R-33865 on the soil microbes which was slight even at 10 mg/l concentration (Nagy et al., 1986).

We measured large differences in the case of the decomposition of EPTC which depended on the microbial activity of soil. This microbial decomposition of EPTC was affected by the number of herbicide exposures, the inoculation with herbicide degrading bacterial strains, microbicide handlings and the type of soil.

ACKNOWLEDGEMENTS

We are grateful to Dr B. Kadlicsko for supplying soil samples with 16 years previous exposure to EPTC from the plot of Res. Group of Putnok of the Res. Inst. of the Univ. of Agric. Sci., Godollo.

REFERENCES

Audus, L.J. 1951 The biological detoxification of hormone heribicides in soil. Plant and Soil III, 170-192.

Capper, B.E. 1982 Herbicide extenders: A new concept in wood control. Proceedings of the New Zealand Weed and Pest Control Conference 35, 222-225.

Fryer, J.D.; Kirkland, K. 1970 field experiments to investigate long-term effects of repeated applications of MCPA, tri-allate, simazine and linuron: Report after 6 years. Weed Research 10, 133-158.

Gray, R.A.; Weierich, A.J. 1968 Behaviour and persistence of thiocarbamate herbicides in soils under different environmental conditions. Proceedings 9th British Weed Control Conference 94-101.

Kaufman, D.D.; Kearney, P.C. 1976 Microbial transformations in the soil. In: Audus, L.J. (Ed.) Herbicides; Physiology, Biochemistry, Ecology, London, Academic Press. pp. 29-64.

Lee, A. 1984 EPTC (S-ethyl N, N dipropylthiocarbamate) - degrading microorganisms isolated from a soil previously exposed to EPTC. Soil Biology and Biochemistry

Lee, A.; Rahman, A.; Holland, P.T. 1984 Decomposition of the herbicide EPTC in soils with a history of previous EPTC applications. New Zealand Journal of Agricultural Research 27, 201-206.

Nagy, I.; Nagy, J.; Geiger, M.; Kecskes, M. 1986 Extenderek mellekhatasa nehany talaj mikroorganizmusra. A Mezogaz-dasag Kemizalasa, Keszthely 97-102.

Obrigawich, T.; Wilson, R.E.: Martin, A.R.; Roeth, F.W. 1982 The influence of temperature, moisture and prior EPTC application on the degradation of EPTC in soils. Weed Science 30, 175-181.

Rahman, A.; Atkinson, G.C.; Douglas, J.A.; Sinclair, D.P. 1979 Eradicane causes problems. New Zealand Journal of Agriculture 139 (3), 47-49.

Rahman, A.; Burney, B.; James, T.K. 1981 Control of grass weeds in maize fields showing decreased activity of EPTC + 25788. Proceedings of the New Zealand Weed and Pest Control Conference 34, 176-181.

Rahman, A.; James, T.K. 1983 Decreased activity of EPTC + R-25788 following repeated use in some New Zealand soils. Weed Science 31, 783-789.

SULFONYLUREA HERBICIDE SOIL RELATIONS

E. M. BEYER, H. M. BROWN & M. J. DUFFY

E. I. du Pont de Nemours & Co., Inc. Agricultural Products Department, Experimental Station, Wilmington, DE 19898, USA

ABSTRACT

Sulfonylureas degrade under field conditions at rates similar to, and often faster than conventional herbicides. Chemical hydrolysis and microbial breakdown are the principal modes of degradation. In acidic soils, chemical hydrolysis is the main degradation pathway while in alkaline soils, where rates of chemical hydrolysis are minimal, microbial breakdown predominates. Breakdown is generally the fastest in warm, moist, light-textured, low pH soils and slowest in cold, dry, heavy, high pH soils. The sometimes long residual activity that has been observed by replanting highly sensitive crops such as sugar beets into sulfonylurea-treated soil is largely due to the extreme sensitivity of these rotational crops and not to an inherently slow rate of breakdown. Soil mobility of a particular sulfonylurea herbicide generally increases with increasing soil pH and decreasing organic matter. As a class, the sulfonylureas are characterized as relatively mobile compounds, and depending on rainfall, net soil water movement and degree of soil drainage, this mobility can be important. However, bensulfuron methyl binds more tightly to soil than do most sulfonylureas resulting in a less mobile and less soil active compound. Within the sulfonylurea chemistry, compounds have been found which undergo very rapid breakdown in soil. Examples include Harmony® (DPX-M6316), which undergoes very rapid breakdown by soil microbes, and Express® (DPX-L5300), which undergoes very rapid chemical hydrolysis in soil. These second generation, short residual products offer substantially greater rotational crop flexibility than many of the earlier sulfonylurea products.

INTRODUCTION

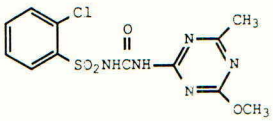
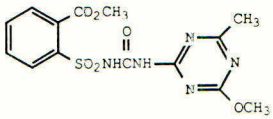
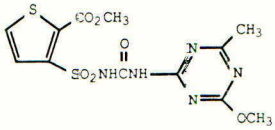
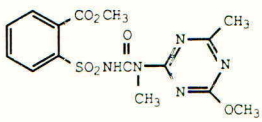
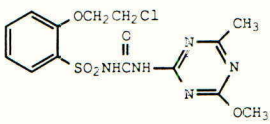
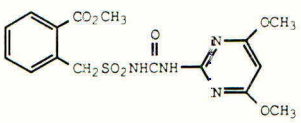
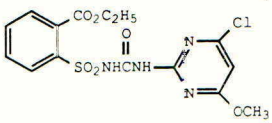
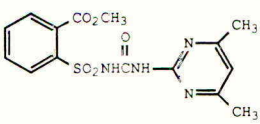
The sulfonylurea herbicides, discovered in 1975 by Dr. George Levitt of Du Pont, have emerged as a major new class of herbicides and an important advance in chemical weed control technology. Worldwide, a total of eight sulfonylurea herbicides have been commercialized (Table 1) and by the mid-1990's it is anticipated that this number could double. With their unprecedented herbicidal activity, use rates have dramatically fallen resulting in application rates of grams rather than kilograms per hectare. Today, a product like Ally® can be applied annually for over 200 years at its recommended use rate of 2 to 8 g/ha before the total dose would equal a single application of many traditional materials now being used at 1 to 4 kg/ha. The need for such broad spectrum, low dosage compounds with greater crop selectivity are important factors contributing to the rapid success of the sulfonylurea herbicides. Equally important are their very favorable toxicological and environmental properties. The acute oral LD_{50} for sulfonylureas in rats is greater than 4000 mg/kg. By comparison, the acute oral LD_{50} of common table salt is 3000 mg/kg.

TABLE 1

Commercialized sulfonylurea herbicides.

TABLE I

SULFONYLUREA HERBICIDES

CHEMICAL STRUCTURE	COMMON/TRADE NAME	PRIMARY USE	APPLICATION RATE (G AI/HA)
	Chlorsulfuron Glean® (Du Pont)	Cereals	4-26
	Metsulfuron Methyl Ally®/Allie®/ Gropper® (Du Pont)	Cereals	2-8
	DPX-M6316 Harmony® (Du Pont)	Cereals	10-35
	DPX-L5300 Express® (Du Pont)	Cereals	5-30
	Triasulfuron Logran® (Ciba-Geigy)	Cereals	10-40
	Bensulfuron Methyl Londax® (Du Pont)	Rice	20-75
	Chlorimuron Ethyl Classic® (Du Pont)	Soybeans	8-13
	Sulfometuron Methyl Oust® (Du Pont)	Noncrop	70-840

Over the past decade, work at Du Pont has concentrated on systematically optimizing the herbicidal activity, crop selectivity and soil residual properties of these molecules. This report, and the one which follows, highlights certain aspects of the soil residual and dissipation properties of these herbicides. For information concerning other aspects, such as mode-of-action and basis of crop selectivity, the reader is referred to a review entitled, 'Sulfonylurea Herbicides' to appear this year in Herbicides: Chemistry, Degradation and Mode of Action, edited by P. C. Kearney and D. D. Kaufman, Vol. 3, Chapter 22, pages 117-189. Marcel Dekker, Inc., New York, NY.

For some time, Du Pont has had a significant commitment to sulfonylurea soils research. The goals of this research are to: (a) develop a fundamental understanding of the key factors important in dissipation and recropping injury, (b) design sulfonylureas with maximum rotational crop flexibility and (c) develop the assay and computer modeling technology needed for defining the soil, weather and recropping patterns where specific sulfonylureas fit best. Significant progress has been made in all of these areas as discussed below. In many instances, other workers have significantly contributed to this progress, especially in areas (a) and (c) above. While only selected references have been cited in this report, a more extensive survey of these contributions can be found in the review cited above. This report will highlight research progress in (a) and (b) above while the subsequent contribution will focus on recent developments in the area of sulfonylurea assay and computer modeling technology.

KEY DISSIPATION PATHWAYS

Chemical Hydrolysis

A major route of sulfonylurea degradation in soil is via chemical hydrolysis of the sulfonylurea bridge. As shown in Fig. 1, sulfonylurea herbicides are weak acids with pK_a's ranging from 3.3-5.2. The neutral form of the sulfonylurea bridge is especially susceptible to hydrolysis, yielding the herbicidally-inactive sulfonamide and amino heterocyclic halves of the parent molecule. Thus, aqueous hydrolysis of sulfonylureas is much more rapid under acidic conditions where a greater proportion of the molecules are in the neutral, hydrolytically susceptible form.

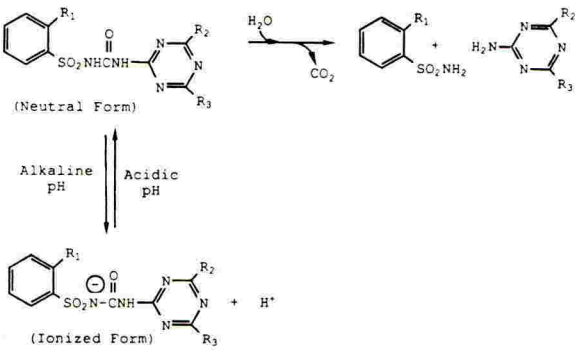


Fig. 1. Sulfonylurea ionization and hydrolysis in water and soil.

The strong influence of pH on sulfonylurea soil degradation is clearly illustrated in Fig. 2. In the ethylene oxide sterilized soils, where microbial degradation is not a factor (top curve in each box), the rate of breakdown by chemical hydrolysis is 15 times faster in the acidic soil (pH 5.9) than the alkaline soil (pH 8.0). The same trend of slower breakdown with increasing pH was observed by Fredrickson and Shea (1986). In a non-sterile, acidic soil (Sharpsburg silty clay loam, pH 5.6, 2.4% organic matter) the degradation half-life of chlorsulfuron at 25°C increased from 1.5 weeks to more than 9 weeks after it was amended with calcium carbonate to increase the pH to 7.5.

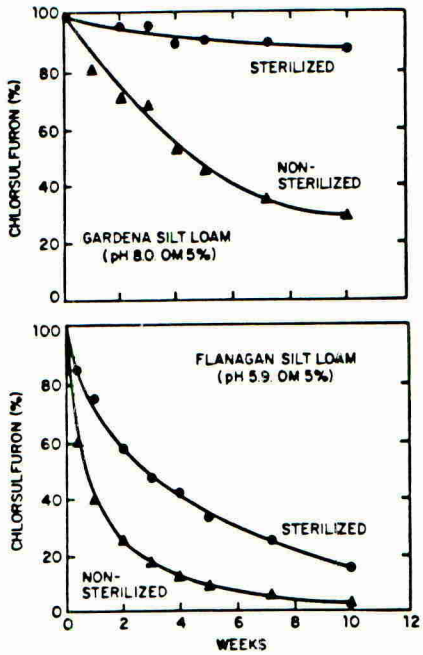


Fig. 2. Chlorsulfuron degradation in sterilized and non-sterilized soils at 30°C (Joshi et al. 1985).

These and numerous related laboratory and field studies have established that chemical breakdown via hydrolysis can be a major factor in the soil dissipation of sulfonylureas. In acidic soils this component is generally a major contributor to total breakdown, whereas in alkaline soils, this component tends to be of minimal importance. To put this in perspective, a single application of 20 g/ha of chlorsulfuron made in the spring of 1982 to an acidic soil (pH 6.3) was found to have no effect the following year on highly sensitive rotational crops. However, in an adjacent area where the soil pH was 7.8, but other conditions were very similar, injury to highly sensitive

crops was observed for the following three years (unpublished results). Thus, soil pH and its influence on sulfonylurea chemical hydrolysis has emerged as one indicator for labeling recrop intervals for sulfonylureas with residual activity.

Microbial Degradation

Soil microorganisms also play a very important role in the degradation of sulfonylureas as seen in Fig. 2. Degradation was significantly faster in the non-sterile, microbially-active soils than in soils that had been sterilized. Over the ten week sampling period, microbial breakdown accounted for 79% of the degradation in the non-sterile Flanagan silt loam and 91% in the alkaline Gardena silt loam. Numerous microorganisms including actinomycetes, fungi and bacteria have been isolated from soil and were shown to actively metabolize sulfonylureas in pure culture (Joshi et al. 1985). Also, degradation is generally faster in freshly obtained soil samples as compared to soil that has been stored for extended periods.

As expected, warm, moist soil conditions which promote microbial activity also promote sulfonylurea degradation. The effect of temperature on chlorsulfuron degradation in non-sterile and sterilized soils is shown in Table 2. In both acidic and alkaline soils, the first half-life decreases 2 to 4-fold as the temperature is increased from 20°C to 30°C. Increasing temperature promotes both chemical hydrolysis and microbial degradation. Also, note again the marked effect of pH on sulfonylurea soil degradation.

TABLE 2

Effect of temperature on chlorsulfuron soil degradation under laboratory conditions (Joshi, Brown and Van, Du Pont - unpublished results).

Temperature (°C)	Rochelle, IL Silt Loam [pH = 5.7; 4.9% OM]		Fargo, ND Silt Loam [pH = 7.5; 5.7% OM]	
	Sterile	Non-Sterile	Sterile	Non-Sterile
	(First Half-Life, Weeks)		(First Half-Life, Weeks)	
30	4.3	1.2	33.0	7.8
20	14.0	4.0	69.0	32.0

Mobility

The soil mobility of sulfonylurea herbicides has been extensively studied and correlated with soil and compound properties. Soil thin-layer chromatography R_f values (Table 3) show that sulfonylureas range from those with low mobility and tight soil binding like bensulfuron methyl to those that are relatively mobile like chlorsulfuron and metsulfuron methyl.

TABLE 3

Soil TLC behavior of sulfonylurea herbicides (T. M. Priester, Du Pont - unpublished results).

Compound	Soil Thin-Layer Chromatography R_f Value*			
	Woodstown Sandy Loam (pH 6.6, OM 1.1%)	Cecil Sandy Loam (pH 6.5, OM 2.1%)	Flanagan Silt Loam (pH 5.4, OM 4.3%)	Keyport Silt Loam (pH 5.2, OM 7.5%)
Chlorsulfuron	0.90 (5)**	0.65 (4)	0.59 (3)	0.52 (3)
Metsulfuron Methyl	0.88 (4)	0.74 (4)	0.70 (4)	0.58 (3)
DPX-M6316	0.92 (5)	0.73 (4)	0.44 (3)	0.49 (3)
Sulfometuron Methyl	0.84 (4)	0.59 (3)	0.26 (2)	0.21 (2)
Chlorimuron Ethyl	0.71 (4)	0.59 (3)	0.41 (3)	0.18 (2)
Bensulfuron Methyl	0.46 (3)	0.30 (2)	0.06 (1)	0.05 (1)

* R_f = Distance of Compound Migration / Distance of Solvent Front ; high values indicate mobility.

** U.S. EPA Classification: (1) immobile, (2) low mobility, (3) intermediate mobility, (4) mobile and (5) very mobile.

These data also illustrate that soil pH and organic matter influence the mobility of sulfonylureas. In general, sulfonylurea soil mobility increases with increasing soil pH and decreasing soil organic matter. Like chemical hydrolysis, these results are related to the ionizable character of these compounds. Table 4 presents the pK_a values for 6 sulfonylureas and shows the dramatic effect of pH on octanol/water partition coefficient (lipophilicity) and water solubility.

TABLE 4

Effect of pH on partition coefficient and water solubility of sulfonylurea herbicides.

	Dissociation Constant (pK_a)	Partition Coefficient (octanol-water, at 25°C)		Water Solubility (ppm at 25°C)	
		pH 5	pH 7	pH 5	pH 7
Chlorsulfuron	3.6	5.5	0.046	60	7000
Metsulfuron Methyl	3.3	1.0	0.014	1100	9500
DPX-M6316	4.0	3.3	0.027	260	2400[a]
Sulfometuron Methyl	5.2	15	0.31	8	70
Chlorimuron Ethyl	4.2	320	2.3	11	1200
Bensulfuron Methyl	5.2	155	4.1	2.9	120

[a] at pH 6

The neutral form of the sulfonylurea molecule (see Fig. 1) is much more lipophilic (less water soluble) than the ionized form. Thus, at pH 5, where a greater proportion of the molecules are in the neutral, undissociated state, the lipophilicity is highest (higher partition coefficient) and the water solubility lowest. Increasing the pH to 7 not only increases the water solubility by 10 to 100-fold but also has a similar dramatic effect on reducing lipophilic character. Consequently, soil pH has a very dramatic effect on overall soil mobility. As the soil pH increases, the equilibrium shifts to favor the more water-soluble ionized form, moving more of the compound from the bound fraction to the soil water phase where it is free to travel with net soil water movement.

In addition to percent organic matter and pH, other soil factors (e.g., porosity, soil type) and environmental factors (e.g., rate of compound application, time of the year, rainfall and soil temperature) can also markedly influence mobility. Using a column packed with a silt loam soil, Nilsson (1985) found that chlorsulfuron moved vertically with rising capillary water. Therefore, during periods of net upward flow of soil water, relatively mobile compounds like chlorsulfuron might reenter the root zone from deeper in the soil profile where it had penetrated during earlier periods of net downward water flow. Our computer model simulations of chlorsulfuron in the U.K. also predict such behavior where net water movement is upward in the spring/summer months in the eastern part of England. Despite the relatively high mobility of some sulfonylureas under certain soil and rainfall conditions, they are very unlikely to pose groundwater contamination problems because of their exceptionally low use rates, low toxicities, and their relatively rapid soil degradation properties.

DISSIPATION UNDER FIELD CONDITIONS

Sulfonylurea degradation under field conditions occurs at rates that are similar to, and often faster than, conventional soil-active herbicides. Palm et al. (1980), when summarizing world-wide field experience, reported that the first half-life of chlorsulfuron in soil is usually between 4 and 8 weeks. For example, under normal growing conditions in Newark, DE, on a Keyport silt loam soil (pH 6.0, 1.4% organic matter), chlorsulfuron was found to have a half-life of about 4 weeks which is comparable to that of metribuzin, somewhat shorter than that of linuron, and about one-half that of bromacil and diuron at the same location.

The disappearance of chlorsulfuron under field conditions in the spring was studied at fourteen locations by J. C-Y. Han and C. Rapisarda (Du Pont - unpublished data). Initial half-lives ranged from 2 to 13 weeks with the slowest rates of degradation occurring in the highest pH soils. Metsulfuron methyl has been shown to degrade at comparable or slightly faster rates than chlorsulfuron under field conditions (Doig et al. 1983, Anderson 1985, Royrvik 1981).

As expected, field degradation of sulfonylurea herbicides in soil has been found to be fastest in warm, moist, light-textured, low pH soils, and slowest in cold, dry, heavy, high pH soils. It must be

emphasized that the 4 to 8 week half-life values that have been reported for chlorsulfuron and metsulfuron methyl refer to only the first half-life. Moreover, this value is frequently determined following an application made during the growing season when conditions are most favorable for initial breakdown. Under harsh environmental conditions (e.g., low rainfall and temperatures) or during subsequent years these half-lives can be longer, as is typical for most other compounds in soil. Therefore, it is important to define the conditions under which the measurement has been made. Soil depth is also important since microbial activity often declines below the plow layer.

Despite the relatively rapid soil dissipation of chlorsulfuron and metsulfuron methyl under favorable growing conditions, there have been reports of extended weed control (O'Sullivan 1982) and injury to sensitive following crops under a variety of soil, weather and recropping practices (Foy and Mersie 1984, Brewster and Appleby 1983, Peterson and Arnold 1985). This residual activity is largely due to the unprecedented sensitivity of certain crops and weeds to these herbicides. As seen in Fig. 3, Sweetser et al. (1982) found wheat to be at least 1000 times more tolerant to chlorsulfuron in soil than extremely sensitive plants such as sugar beet. For example, sugar beet root growth can be affected by concentrations of chlorsulfuron and metsulfuron methyl of less than 1 ppb, although soil factors such as organic matter can markedly shift this threshold sensitivity value. In contrast to most other herbicides, where dissipation of 80% of the applied material is normally adequate for rotational crop safety, 99% of the applied chlorsulfuron (>6 half-lives) must dissipate in some situations in order to drop below the threshold soil concentration that can cause injury to such highly sensitive rotational crops. Thus, recrop intervals for sulfonylurea herbicides result from the influence of soil, weather and compound related properties on dissipation coupled with the relative sensitivity of the desired rotational crop.

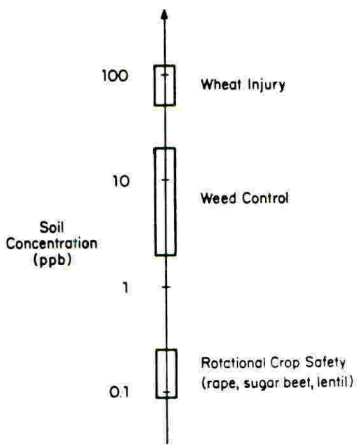


Fig. 3. Schematic representation of relative crop and weed sensitivity to chlorsulfuron in a sandy loam soil, pH 6.5.

SHORT RESIDUAL SULFONYLUREAS

Significant progress has been made in modifying sulfonylurea chemistry to accelerate soil degradation. This is illustrated in Fig. 4 where the degradation rates of the new, short residual cereal herbicides, DPX-M6316 and DPX-L5300 are compared to the established cereal herbicides, chlorsulfuron and metsulfuron methyl. Under identical conditions, DPX-M6316 and DPX-L5300 degrade at initial rates 20 to 50 times faster than chlorsulfuron and metsulfuron methyl. Worldwide recropping studies have confirmed the more rapid dissipation and excellent rotational crop flexibility of these new compounds.

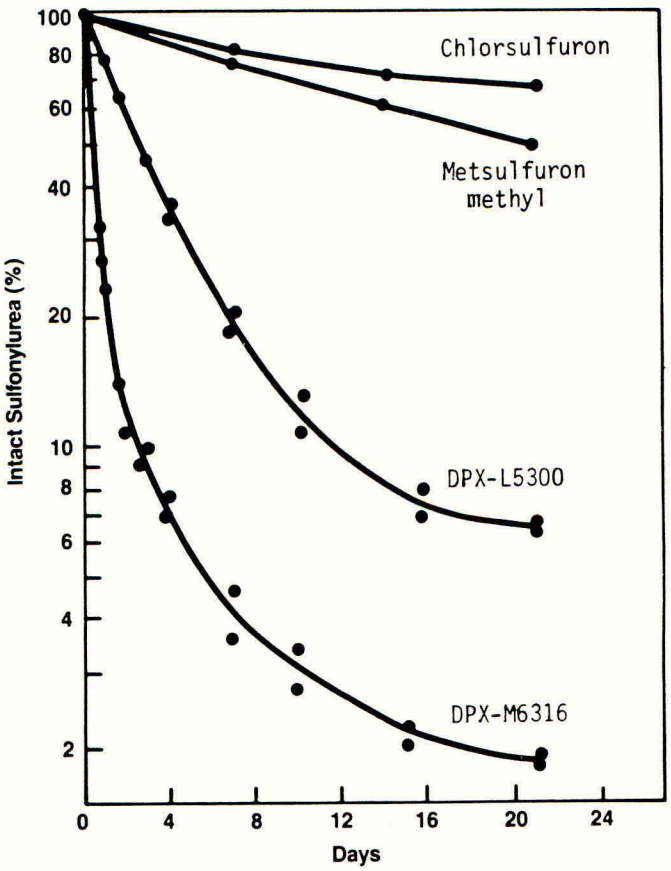


Fig. 4. Relative soil degradation of four sulfonylurea herbicides in non-sterile Gardena silt loam (pH 8.0, 5% OM) at 30°C and 1.0 bar moisture.

The structures for these four cereal herbicides are shown in Table 1. DPX-L5300 is identical to metsulfuron methyl except for the methyl group on the bridge nitrogen. Because of this modification the compound is much more susceptible to chemical hydrolysis. Even in alkaline soils this compound undergoes rapid hydrolysis although alkalinity does have a moderating effect. DPX-M6316 differs from metsulfuron methyl by having a thiophene rather than a phenyl ring. While this change has little effect on chemical hydrolysis, DPX-M6316 is much more susceptible to microbial degradation (Brown et al. 1987).

These recent advances in chemistry and understanding open the way to even more versatile sulfonylurea herbicides for solving many of the world's existing weed problems. With their many attributes, the sulfonylurea herbicides are proving to be a very important advancement in weed control technology.

REFERENCES

Anderson, R.L. (1985) Environmental effects of metsulfuron methyl and chlorsulfuron bioactivity in soil. Journal of Environmental Quality 14, 517-521.

Brewster, B.D.; Appleby, A.P. (1983) Response of wheat (Triticum aestivum) and rotation crops to chlorsulfuron. Weed Science 31, 861-865.

Brown, H.M.; Joshi, M.M.; Van, A.T. (1987) Rapid soil microbial degradation of DPX-M6316. WSSA Abstracts 27, 75.

Doig, R.I.; Carraro, G.A.; McKinley, N.D. (1983) DPX-T6376 - A new broad spectrum cereal herbicide. Proceedings of the British Crop Protection Conference, 3, 1-8.

Foy, C.L.; Mersie, W. (1984) Chlorsulfuron selectivity and persistence in small grains. Proceedings of the Southern Weed Science Society 37, 108.

Fredrickson, D.R.; Shea, P.J. (1986) Effect of soil pH on degradation, movement and plant uptake of chlorsulfuron. Weed Science 34, 328-332.

Joshi, M.M.; Brown, H.M., Romesser, J.A. (1985) Degradation of chlorsulfuron by soil microorganisms. Weed Science 33, 888-893.

Nilsson, H. (1985) Persistence and mobility of chlorsulfuron in arable soils. Investigations 1982-1983. Weeds Weed Control 26th Swedish Weed Conf. 76-81.

O'Sullivan, P.A. (1982) Response of various broadleaved weeds and tolerance of cereals, to soil and foliar applications of DPX-4189. Canadian Journal of Plant Science 62, 715-724.

Palm, H.L.; Riggleman, J.D.; Allison, D.A. (1980) Worldwide review of the new cereal herbicide - DPX-4189. Proceedings of the British Crop Protection Conference-Weeds 1, 1-6.

Peterson, M.A.; Arnold, W.E. (1985) Response of rotational crops to soil residues of chlorsulfuron. Weed Science 34, 131-136.

Royrvik, J. (1981) Degradation studies with DPX-4189 in clay test location: Flakkebjerg. Nordic Plant Protection Conference, Uppsula, 2-5.

Sweetser, P.B.; Schow, G.S.; Hutchison, J.M. (1982) Metabolism of chlorsulfuron by plants; biological basis for selectivity of a new herbicide for cereals. Pesticide Biochemistry and Physiology 17, 18-23.

PREDICTING SULFONYLUREA HERBICIDE BEHAVIOR UNDER FIELD CONDITIONS

M. J. DUFFY, M. K. HANAFEY, D. M. LINN, M. H. RUSSELL & C. J. PETER

E. I. du Pont de Nemours & Co., Inc., Agricultural Products Dept., Stine/Haskell, P.O. Box 30, Newark, DE 19714, USA

ABSTRACT

The dissipation of sulfonylurea herbicides has been extensively studied at locations throughout the world. Together with the many laboratory and greenhouse experiments aimed at understanding sulfonylurea soil relations, these data have led to the development of a computer model capable of simulating the behavior of sulfonylurea herbicides in the field. Use of this model to simulate herbicide behavior in the U.K. following an autumn application of 15-20 g/ha chlorsulfuron has provided important insight into why, under certain conditions, sensitive crops like sugar beet cannot be safely planted. Determining exactly when such sensitive crops can be safely planted into treated fields has been aided by the development of a highly sensitive bioassay. In this procedure, lentils are grown under controlled environmental conditions in soil samples taken from treated fields. After several weeks, visual injury ratings are made and used to predict the potential for damage to a given rotational crop. Sugar beet injury projections made using this method to assay field samples were highly correlated with actual sugar beet damage observed in the field.

INTRODUCTION

Establishing the best fit for sulfonylurea herbicides in the marketplace by understanding the influence of soil, weather and recropping patterns has been a major goal of Du Pont's commitment to soils research. An extensive, world-wide field program involving hundreds of locations is at the core of this effort. Equally important are the many laboratory and greenhouse studies aimed at defining the behavior of sulfonylurea herbicides in the soil. These research thrusts have led to recrop intervals which are shortest in regions of warm, moist, light-textured, low pH soil where sulfonylurea herbicide breakdown is fastest. By contrast, rotational crop intervals are longer in cold, dry, heavy-textured, high pH soils where dissipation is slower.

While field recrop tests provide an accurate measure of the duration of biological activity of a compound, they do have some disadvantages. For example, it is impractical to test under all possible combinations of soil, weather and recropping practices; results can often take longer than a year to obtain and the tests themselves are time consuming and costly to conduct. Therefore, reducing the number of field tests that must be performed to reliably predict recrop

intervals is an important business objective. Two approaches can be envisioned. Either the residual level of herbicide in the soil can be assayed prior to recropping and then used to assess the risk of injury to sensitive following crops, or the behavior of the herbicide in the field can be simulated by a suitable mathematical model that, given appropriate input data, can be run on a computer to predict injury for any desired scenario. This report will highlight the recent progress that has been made in the assay of residual levels of sulfonylurea herbicides and in the development of predictive mathematical models.

ASSAY OF SULFONYLUREA HERBICIDE SOIL RESIDUES

To predict the impact that residual levels of herbicide may have on a rotational crop, soil samples are obtained from fields previously treated with chlorsulfuron and/or metsulfuron methyl and analyzed prior to recropping. The assay results can be translated into an estimate of following crop injury providing an adequate data base exists which correlates measured residual levels of chlorsulfuron with actual field recrop injury. The challenge of this approach is to develop an assay that is sensitive enough to detect the extremely low residual levels in soil and then to correlate this with the wide range of crop sensitivities.

With regard to assay development, two approaches are possible - analytical and biological. The analytical procedure that has been developed for determining chlorsulfuron residues in soil (Zahnow 1982) uses normal-phase liquid chromatography with photoconductivity detection for the final measurement. Detection limits of 0.2 parts-per-billion have been demonstrated, but conducting routine analyses at these levels is very difficult. Similar HPLC methods are available for metsulfuron methyl and sulfometuron methyl. Beginning with the work of Kelley et al. (1985), Du Pont has continued to develop enzyme-linked immunosorbent assay (ELISA) technology for the determination of chlorsulfuron soil residues. When sensitive rotational crops, e.g., sugar beet, are planted into fields in which chlorsulfuron is detected by either of these methods, injury to that crop will very likely be observed.

Despite their relatively low detection limits, there have been instances where certain highly sensitive crops have been injured by chlorsulfuron residues that were not detected by these analytical methods. Therefore, more sensitive biological techniques have been developed. One such procedure uses lentil, a highly sensitive crop, as the indicator species of a bioassay method for detecting chlorsulfuron and metsulfuron methyl in soil. In this procedure, lentils are planted in the soil to be assayed. The pots are placed in a growth chamber maintained at 75°F during a 16 hour photoperiod and 65°F at night. Pots are watered upon demand twice daily. After 21 days, the soil is gently washed from the roots and a visual injury rating is made versus an untreated control. When making an injury rating, the length and appearance of the secondary roots is more

significant than it is for the primary roots. Residues as low as 0.01 parts-per-billion, well below the threshold of injury to sensitive rotational crops, have been measured by this Laboratory Recrop Bioassay. Crops are uninjured when grown in fields from which soil samples have not shown injury in this bioassay. In those cases where injury is observed in the laboratory, empirical correlations have been established which allow the accurate prediction of the risk of injury to sensitive rotational crops grown in the field.

MODELING THE BEHAVIOR OF SULFONYLUREA HERBICIDES

The development of a predictive mathematical model capable of simulating the degradation and movement of sulfonylurea herbicides in the soil as a function of time is a formidable challenge. Three general types of information are required to the dissipation of a compound under field conditions. It is necessary to have information about soil characteristics, weather patterns and properties of the compound and its interaction with the soil as the environment changes from the time of application to recropping. If the goal of the dissipation simulation is to assess the risk of injury to a sensitive rotational crop, the problem is further complicated by the need for a plant response model which is capable of translating the nonuniform distribution of residual herbicide in the soil to a prediction of following crop response. Considering the inherent complexity of this problem, and the stage of development of this technology, it is obvious that any model is of necessity going to be a gross simplification of the real world. Despite the difficulty of this problem, Walker (1987) and Nicholls et al. (1982, 1983) have successfully demonstrated the ability to model the chemical dissipation of several herbicides, including chlorsulfuron (Walker and Brown, 1983), under U.K. conditions.

We too, have sought to develop such a model. The ultimate goal of which is to be able to assess the potential for injury to sensitive rotational crops following application of sulfonylurea herbicides, especially chlorsulfuron. Our model contains a mathematical description of three conceptually independent processes: (1) variation of soil water content and soil temperature over time from the soil surface to the water table, (2) herbicide behavior in the soil (degradation, sorption, leaching, etc.), and (3) plant response to the herbicide. Although physical and biological science forms the core of the model, a great deal of additional software is layered around this core to provide for easy and flexible specification of model inputs, and presentation of the vast amount of model outputs in an understandable, graphical format.

Variations of soil water content and soil temperature are described by partial differential equations. The water table (saturated, and at constant temperature) is the lower boundary condition, while the soil/atmosphere interface is the upper boundary condition.

The latter is more complicated, and more important, for it is through this interface that the soil is coupled to the weather. Daily rainfall and evapotranspiration are required inputs. If these data are not available, a statistically valid sequence of daily rainfall can be generated from monthly total values by a stochastic weather simulator and potential evapotranspiration can be computed from air temperature, humidity, wind speed, and net solar radiation via the Penman equation. Soil hydraulic parameters are computed from textural class. Soil heat conductivity is calculated from soil water potential, with the heat capacity depending on moisture content. The model includes a description of the freeze/thaw cycle, which has a drastic effect on hydraulic conductivity, and a big effect on soil temperatures because the latent heat of fusion is much larger than the heat capacity.

An important feature of the degradation curves obtained from studies with sulfonylurea herbicides in non-sterile (microbially active) soils conducted in our laboratories is the biexponential character of the breakdown process. This behavior is characterized by an initially rapid decay followed by a period of slower degradation. For chlorsulfuron, the transition to the slower breakdown phase occurs after 2 to 4 weeks. In sterilized (microbially inactive) soil, the degradation appears to follow first order kinetics. One way of explaining this observed behavior views the herbicide as partitioning between two compartments in the soil (Figure 1). Following application, the compound is in the "available" compartment where it can undergo degradation by both chemical hydrolysis and microbial breakdown. Concurrently, the compound can also move into a "protected" compartment where it is postulated to be unavailable to soil microorganisms. While in the "protected" compartment, chemical hydrolysis continues but microbial degradation stops until the herbicide returns to the "available" compartment.

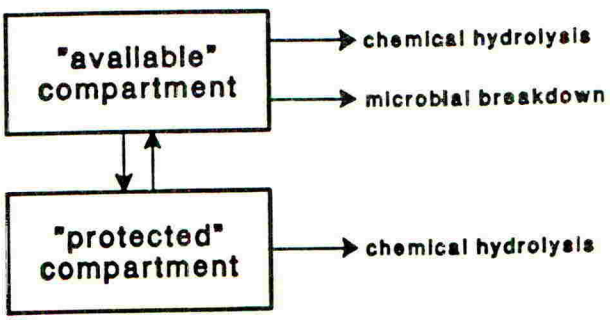


Fig. 1. The two-compartment model of soil degradation.

The mathematical treatment of degradation that is contained in our model makes use of this two compartment description. First order decay takes place at different rates from each compartment, and mass transfer occurs between the compartments. Mass transfer can be modeled either as a diffusion or interface controlled process. In either case, the resulting degradation can be approximated by a biexponential curve. Herbicide transport occurs in the soil water phase by convection, diffusion, and dispersion. This latter term includes the effect of spatial heterogeneity neglected in the one dimensional simulation. Critical to the transport model is the fraction of herbicide in the soil water. In the simplest approach it is assumed that the partitioning between water and soil remains at equilibrium. More realistically this equilibrium state is perturbed by the coupling of the kinetics of sorption, degradation, and soil water flux.

The plant response model is still in the development stage. Currently plant response is computed by averaging herbicide soil water concentration over depth (typically the root zone) and time following recrop. An assessment of the injury potential is obtained from a probit plot that linearly relates percent crop injury and logarithm of herbicide concentration in the soil water.

The complexity of the model and the desire to be able to simulate large numbers of situations makes finding appropriate numerical methods to solve the many equations and designing a computer program with the necessary computational efficiency essential. A stabilized, iterative, Crank-Nicolson scheme was found to reliably solve the differential equations with a reasonable amount of computational effort. The model is currently being run on a Cray 1A supercomputer, where the nearly 5 billion arithmetic operations needed to simulate the fate of a sulfonylurea over a two-year period are performed in less than 2 minutes.

The model was initially validated by simulating recrop injury for over 200 field tests in the United States conducted over a four year period. Application rate, soil properties, recrop interval and weather varied widely among the projections. With continued refinement, good quantitative agreement was eventually obtained between predicted injury and actual recrop injury ratings for the great majority of these validation simulations. The best agreement occurred in cases where observed injury was slight (<20%) or severe (>40%). In cases of intermediate injury, agreement between predicted and actual injury ratings was only fair. On the whole, we believe that the model provides a good assessment of the biological activity of chlorsulfuron residues in the soil.

UNDERSTANDING SUGAR BEET INJURY FROM CHLORSULFURON

In the spring of 1986 and 1987 some sugar beet damage was reported in the U.K. in fields treated with chlorsulfuron in the autumn of 1984 and 1985, respectively. Most of the injury occurred in the eastern

sugar beet counties of Norfolk and Lincolnshire with only a few occurrences in the west midlands (e.g., Shropshire). A comparison of features in areas where injury was observed with uninjured regions showed that the main distinguishing feature was net precipitation, i.e., rainfall minus evaporation. In Norfolk the net annual precipitation was about 30 mm while in Shropshire it was six times higher. The many computer simulations that were run have confirmed the greater risk of injury in the eastern sugar beet counties and helped provide an understanding of why the damage occurred.

Little degradation takes place during the fall and winter months immediately following an autumn application of chlorsulfuron. Because the net movement of water is into the soil profile during this period, and since chlorsulfuron is a relatively mobile compound, it is leached out of the root zone. In the spring and summer months when the direction of water flow in the soil is toward the surface, herbicide can be moved back into the root zone where it can injure sensitive rotational crops. Our simulations show that over a period of years in the U.K., chlorsulfuron continually degrades as it moves up and down in the soil.

Because herbicide leaching is a contributing factor to the dissipation of fall applied products, the drainage characteristics of the soil cannot be ignored. Nicholls (1986) was the first to recognize this with regard to understanding the sugar beet injury from chlorsulfuron. In a preliminary survey of locations where sugar beet injury was reported, he found that 47% of the cases occurred on pleosols and stagnogleys which have impeded drainage, 39% of the cases were on gleys which are intermittently waterlogged, and the remaining 14% were on freely draining soils. Our simulations have confirmed that, at sites having alkaline pH and impeded water flow or a high water table, autumn applications of 15 to 20 g/ha of chlorsulfuron can damage sugar beet more than 1 year after treatment.

The behavior of spring applied sulfonylurea herbicides is markedly different to that described above for autumn treatments. Because of the much lower application rate used in the spring vs. the autumn (5-6 g/ha vs. 15-20 g/ha), and because the flow of soil water allows the compound to remain in the warmer, upper layer of soil where more degradation occurs during the first growing season, the potential for injury to sensitive rotational crops is much less. This is apparent when the time interval to reach safety to sugar beet following 5 g/ha Ally® (metsulfuron methyl) in the spring is compared to that needed after a total of 20 g/ha Finesse® (15 g/ha chlorsulfuron + 5 g/ha metsulfuron methyl) in the autumn. As contrasted to fall applied Finesse®, our simulations predict that there will be an adequate safety margin for sugar beet one year after spring treatments of Ally®. The widespread favorable commercial experience with spring applied Ally®, since its introduction in the U.K., confirms the accuracy of these projections.

REFERENCES

Kelley, M.M.; Zahnow, E.W.; Petersen, W.C.; Toy, S.T. (1985) Chlorsulfuron determination in soil extracts by enzyme immunoassay. J. Agric. Food Chem. 33, 962-965.

Nicholls, P.H.; Walker, A.; Baker, R.J. (1982) Measurement and simulation of the movement and degradation of atrazine and metribuzin in a fallow soil. Pesticide Sci. 12, 123-132.

Nicholls, P.H.; Briggs, G.G.; Evans, A.A. (1983) Simulation of herbicide movement in winter. Aspects of Applied Biol. 4, 485-494.

Nicholls, P.H. (1986) Personal communication.

Walker, A.; Brown, P.A. (1983) Measurement and prediction of chlorsulfuron persistence in soil. Bull. Environ. Contam. Toxicol. 30, 365-372.

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

Zahnow, E.W. (1982) Analysis of the herbicide chlorsulfuron in soil by liquid chromatography. J. Agric. Food Chem. 30, 854-857.

THE BEHAVIOUR OF CHLORSULFURON AND METSULFURON IN SOILS IN RELATION TO INCIDENTS OF INJURY TO SUGAR BEET

P.H. NICHOLLS, A.A. EVANS

Institute of Arable Crops Research, Harpenden, Herts., AL5 2JQ, U.K.

A. WALKER

Institute of Horticultural Research, Wellesbourne, Warwick, CV35 9EF, U.K.

ABSTRACT

The rates of degradation of chlorsulfuron and metsulfuron were measured in five soils of different pH and, for two of them, at three depths. Half-lives were very long (up to 627 days) in soils of pH >7. Sodium azide slowed the rate of degradation of metsulfuron more than that of chlorsulfuron which implies that microbial degradation is more important for metsulfuron. To identify the factors involved in the observed herbicide damage to sugar beet crops, the location of incidents were plotted on soil maps. Most cases occurred on sites with impermeable or intermittantly waterlogged subsoil and only a few on apparently free draining sites. Simulations provided evidence that chlorsulfuron applied in autumn can readily leach into subsoils where both microbial activity and hydrolysis of the herbicide could be slow. At sites where drainage is impeded, the tapping of this subsoil by sugar beet and/or return of this water to the topsoil by capillary action could lead to damage.

INTRODUCTION

Several incidents of injury to sugar beet in the summer of 1986 were caused by chlorsulfuron applied to previous crops of winter wheat in the autumn of 1984. This was perhaps surprising for two reasons. Firstly, because Walker and Brown (1983) had measured the half-life of chlorsulfuron in soil at 10°C to be 2 months and had predicted residues 18 months after an October application to be less than 0.2% of that applied. Secondly, Nicholls and Evans (1985) had shown that chlorsulfuron was very weakly adsorbed by soils of normal pH and so it was expected to be leached below the root zone of the crop during two winters in the field. So *a priori*, incidents of failure to control weeds, when uptake from soil was required, was considered much more likely than incidents of crop injury because of the risk of herbicide being leached away from weed roots. Nevertheless Richardson *et al* (1981) had warned that residues of chlorsulfuron toxic to beet could persist for more than 53 weeks in soil.

Chlorsulfuron is aplied at such low rates that analysis of residues from commercial applications was not possible with conventional instrumental techniques and bioassays had not been sufficiently developed in the U.K. at the time. Therefore simulation was an important method for developing an understanding of the behaviour of chlorsulfuron in field soils. The present work attempts to explain how residues can persist at concentrations which are toxic to sugar beet for up to two years after autumn applications.

MATERIALS AND METHODS

Rates of degradation

The properties of the soils used are given in Table 1. The herbicides were wettable powder formulations (80% ai). All experiments involved soils incubated at 20°C and the water content indicated in the Table. The initial concentration of herbicide was 5.0 mg/kg and residual concentrations were measured by HPLC. The herbicides were extracted from soil in methanol + water (80 + 20 by volume) containing 0.1% acetic acid. The HPLC mobile phase was methanol + water (70 + 30 by volume) containing 0.1% acetic acid. The column used was Lichrosorb RP-18 and detection was at 245 nm. All incubations lasted from 84 to 104 days. In the incubations involving sodium azide, the inhibitor was mixed into soil at 800 mg/kg prior to incorporating the appropriate herbicide.

Adsorption

Adsorption coefficients were measured by the method given in Nicholls and Evans (1985).

Leaching study

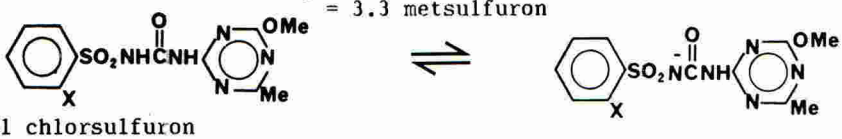
Measurements were made on fallow plots at Rothamsted and Woburn. The properties of the soils were given in Nicholls and Evans (1985) and the experimental method described by Nicholls *et al* (1983). Technical-grade chlorsulfuron was applied at the rate of 420 g ha^{-1} and analysed by the HPLC method given above.

Simulations

Simulations were made using the model CALF as described by Nicholls *et al* (1982), which uses the empirical concept of mobile and immobile water categories. Values for the water content at field capacity and -2×10^{-5} Pa (-2 bar) were 0.29 and 0.17 $\ell\ kg^{-1}$ respectively. Adsorption coefficients are given on the figures. Rates of degradation in the topsoil (0-30 cm) were calculated using the equations of Walker and Barnes (1981) from the coefficients given by Walker and Brown (1983). In order to simulate slow rates of degradation in the subsoil (>30 cm), for the data in Figure 4 and Table 2, rates were arbitrarily set at one tenth of those which would have occurred in topsoil. Measurements of rainfall, evaporation from an open water surface and maximum air temperature were those measured daily at 09.30 at Rothamsted.

PHYSICO-CHEMICAL PROPERTIES

pK_a = 3.7 chlorsulfuron
= 3.3 metsulfuron



X = Cl chlorsulfuron
= $COOCH_3$ metsulfuron

Chlorsulfuron and metsulfuron, when unionised, are moderately polar (log K_{OW} = 1.5 and 0.8 for chlorsulfuron and metsulfuron respectively) but are weak acids being almost completely ionised at normal soil pH (6-8). Adsorption of chlorsulfuron to soil is weak (Fig. 1). Anions are more polar than the unionised molecule by 3-4 log K_{OW} units and because the balance of charge at soil surfaces is negative, anions are more weakly adsorbed than the unionised parent molecule. The consequence of such weak adsorption is a great potential for movement through soil.

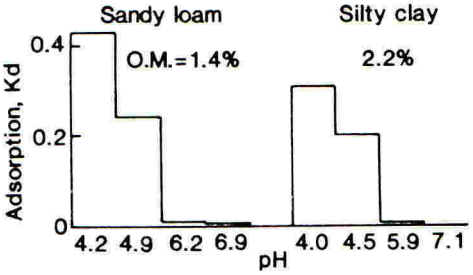


Fig. 1. Adsorption of chlorsulfuron on soils of different pH

DEGRADATION

Degradation of chlorsulfuron in soil is by acid-catalysed hydrolysis so that persistence increases with pH. This is illustrated in Table 1b where, in general, soil half-life increases with soil pH. The relationship is better seen in the depth samples of Soakwaters soil which have similar organic matter content but degradation in the deepest layer which has a high pH, is about 30 times slower than that in the top layer. The rate of degradation from the deepest layer in the Wharf Ground soil was also slow but in this soil, although pH was slightly higher, organic matter content and hence probably microbial activity was low.

Degradation rates for metsulfuron were almost identical to those of chlorsulfuron. Sodium azide, which inhibits microbial activity, increased the persistence of metsulfuron more than that of chlorsulfuron. This indicates microbial activity is more important for the degradation of metsulfuron than of chlorsulfuron.

TABLE 1

Soil properties and rates of degradation of chlorsulfuron and metsulfuron at 20°C. The half-lives shown in parentheses are extrapolations from the observed data since the residual concentration did not fall to 50% of the intial amount during the incubation period. The half-lives marked * were obtained in the presence of the microbial inhibitor sodium azide.

		Organic matter (%)	pH	Water content at 0.33 bar (%)	Half-life in soil (days) Chlorsulfuron	Metsulfuron
(a) Depth samples						
Soakwaters	0-20	2.01	5.8	11.9	21	23
	20-40	1.86	5.6	9.7	22	24
	40-60	1.85	7.5	10.1	(627)	(303)
Wharf Ground	0-20	2.49	6.2	12.8	60	75
	20-40	2.08	6.2	12.4	58	66
	40-60	1.64	6.7	12.2	91	94
(b) Different soils with pH range						
Soakwaters		2.01	5.8	11.9	25 (129)*	29 (420)*
Wharf Ground		2.49	6.2	12.8	73 (149)*	84 (1349)*
Hinton		1.49	6.4	9.9	27 (174)*	32 (111)*
Sutton Scotney		6.66	7.2	32.8	(120) -	75 -
Kirton		3.45	7.8	19.0	(163) (292)*	(190) (1000)*

LEACHING

Chlorsulfuron applied at high rates to the soil surface was leached considerably by the rain that fell in autumn and winter (Figure 2). Concentrations near the surface only one month after application were very small especially for the latest application.

Simulations, using measured values of the adsorption coefficient and degradation coefficients given by Walker and Brown (1983), indicate that the Rothamsted-NVRS model predicted distributions with moderate precision.

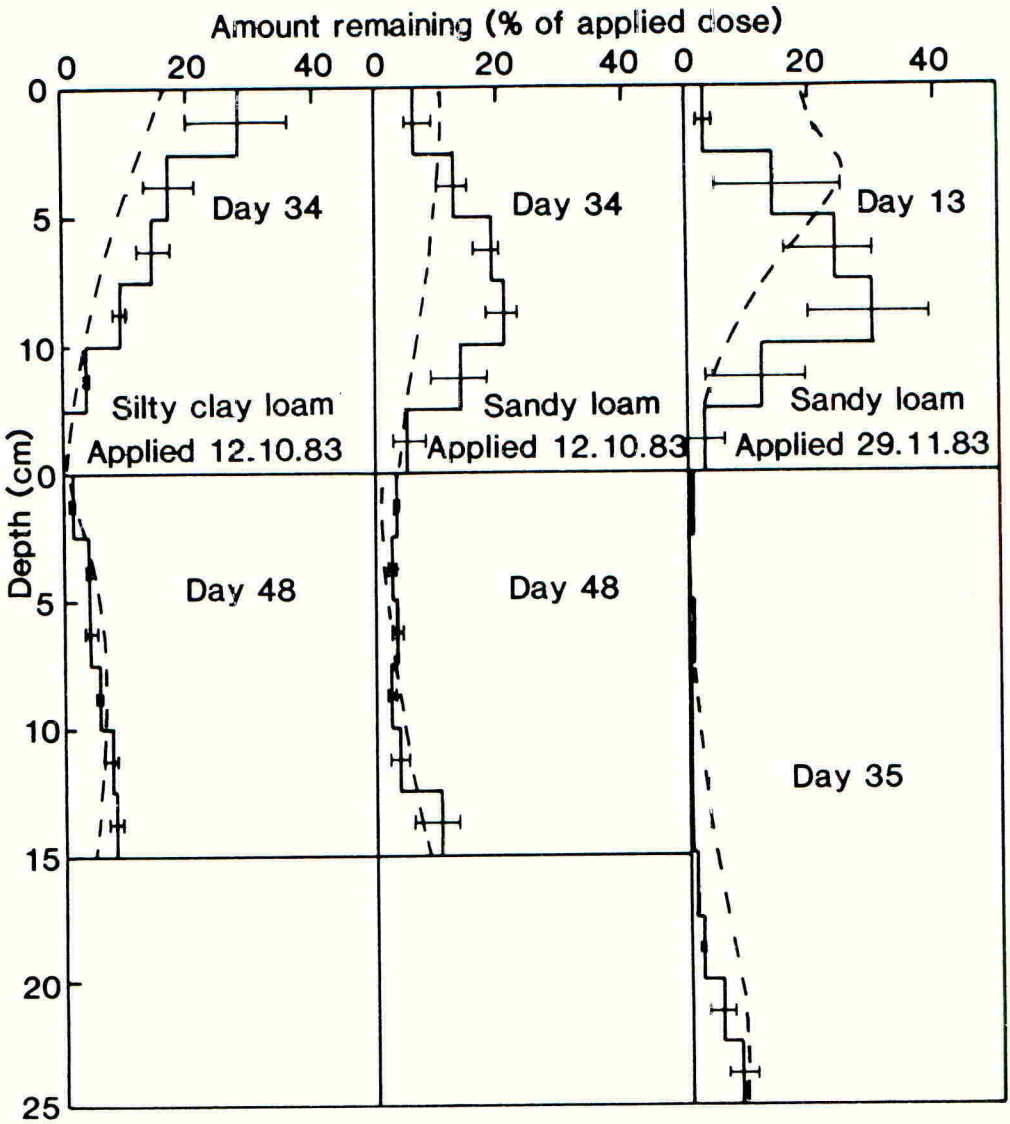


Fig. 2. Distribution of chlorsulfuron: ——— measured values (bars represent range of duplicate measurements), - - - simulated values. Measured values of the adsorption coefficient (K_d) used in the simulation were 0.005 and 0.067 for the sandy and silty clay loam respectively.

WEATHER

The distribution of chlorsulfuron in soil is a product of leaching and degradation, and these in turn are dependent on weather. The data for the weather which occurred after applications in autumn 1984 and 1985 are given in Figure 3. Rainfall occurred randomly throughout the period and so was impossible to predict. However evaporation of water is consistently large in summer and very small in winter. Consequently, rainfall exceeds evaporation in winter enabling leaching to occur whilst in summer evaporation of water exceeds rainfall giving net upward movement of water and of herbicide. Not surprisingly, soil temperature follows a similar seasonal pattern to that of evaporation.

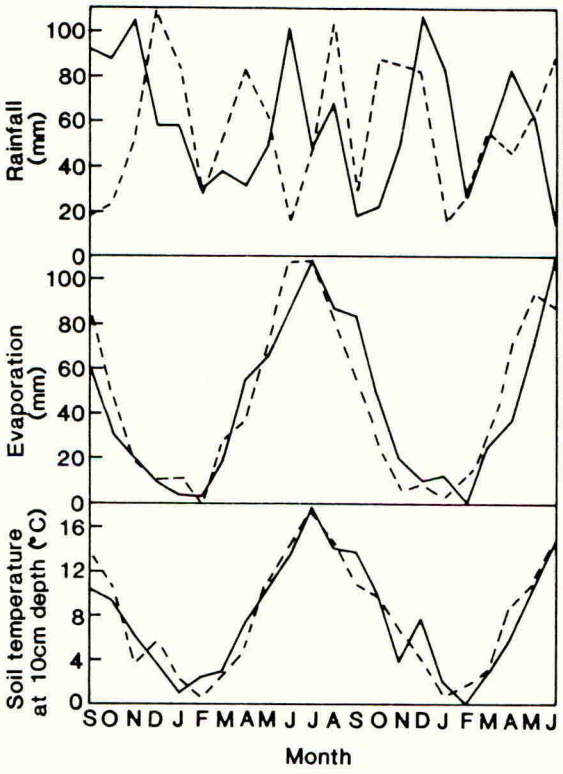


Fig. 3. Weather data. ——— starting September 1984, --- starting September 1985.

SIMULATIONS

Simulations (Figure 4) indicate that in a free draining soil chlorsulfuron might leach to more than one metre depth within 18 months of application. Although sugar beet can root down to one metre depth, damage in these circumstances is difficult to envisage. However, there are three types of drainage in soil profiles:- free draining, intermittantly water logged and impermeable.

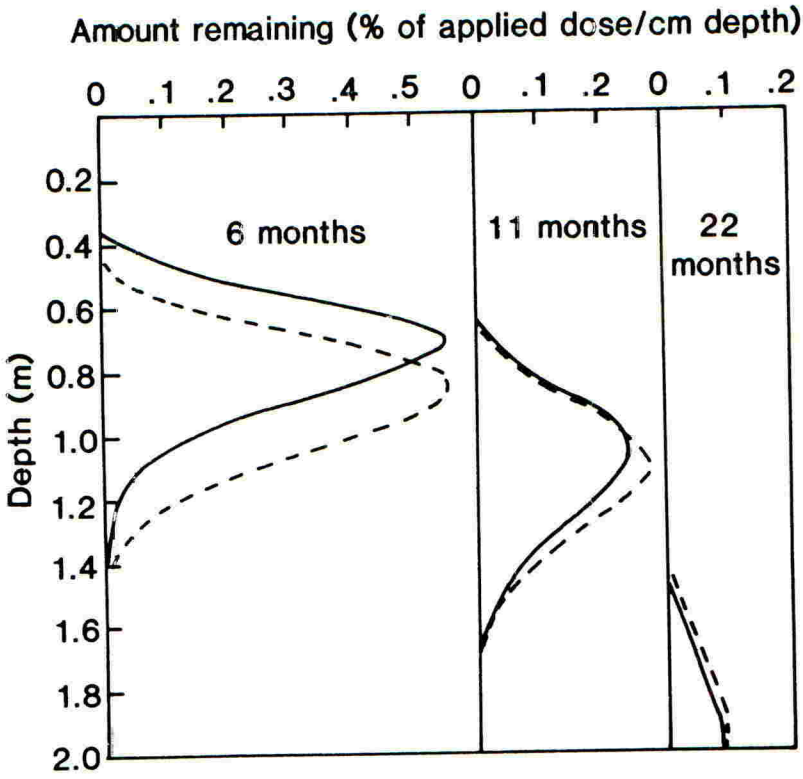


Fig. 4. Simulated distributions of chlorsulfuron using adsorption coefficient K_d = 0.02, ---- applied October 1984, applied October 1985

In an impermeable subsoil, residues will be prevented from leaching by the slow rate of permeation and if that subsoil were of high pH then residues could persist. In an intermittantly waterlogged soil, leaching would become slow as the water table was approached and the hydraulic head became small and there would be the possibility of residues being returned to the surface as soils dried (Phillips 1964). It would clearly be futile to simulate leaching in an impermeable soil but it is important to investigate the persistence of residues where there is a permeable subsoil in which degradation is nevertheless slow. The model CALF was set to calculate rates of degradation in topsoil (<30 cm depth) using rate constants measured by Walker and Brown (1983). Furthermore slow degradation of herbicide in subsoil was modelled by setting rates of degradation of herbicide which had leached below 30 cm depth to only one tenth of those which were set to occur in the topsoil. One tenth is an arbitrary value which lies between the values measured for Soakwaters and Wharf Ground. The simulations (Table 2) indicate that total soil residues are much greater after an autumn application than after spring application. This is because in autumn herbicide can be rapidly leached into subsoil where it can persist because of cooler conditions, high pH and/or lower microbial activity (Anderson and Humburg 1987). After spring applications, residues stay near the surface where higher soil temperatures during summer and high microbial activity favour rapid degradation. Residues from spring applications are therefore quite small by the time winter rain can leach them away.

TABLE 2

Simulation of chlorsulfuron or metsulfuron residues in a silty clay loam assuming adsorption K_d = 0.02

Time after application	Autumn application		Spring application	
	1-10-84	1-10-85	1-4-84	1-4-85
6 months	51	48	10	6
12 months	38	36	6	4

REPORTS OF DAMAGE TO FOLLOWING CROPS

Reports of injury to sugar beet where chlorsulfuron had been used previously were plotted on soil survey maps (Anon 1983) to discern a relationship between sites of injury and the type of subsoil. The results are given in Table 3.

TABLE 3

Subsoil type at location of reported sugar beet damage

		1986	1987
Impermeable	pelosols stagnogleys	80	55
Intermittently waterlogged	gleys	55	60
Clay or clay-enriched subsoil	argillic	6	16
Peat		4	1
Free drained		12	30

The majority of incidents occurred on impermeable or intermittently waterlogged subsoils. Many incidents occurred in the Fens and the Norfolk Broads and in other low lying sites close to rivers or streams. Few incidents were reported from sites with free draining subsoils, such as those in the sugar beet growing areas due north of Bury St Edmunds or on the Lincolnshire or Yorkshire Wolds. Caution must be used in studying these figures because diagnosis of injury and its attribution to sulphonylureas is difficult although the reports were made by independent specialists of the British Sugar Corporation.

CONCLUSIONS

Chlorsulfuron and metsulfuron can persist in soils of high pH and low microbial activity where half-lives of at least 18 months are possible. It is significant that lime is usually applied to fields where sugar beet is grown and often this is done before the preceding cereal crop is sown. This means that these herbicides applied before beet crops will almost inevitably be applied to soils of high pH and hence will have a high probability of persisting.

Chlorsulfuron and metsulfuron are potentially highly mobile in soil and autumn applications are rapidly leached into the subsoil of a free draining profile. Spring applications tend to stay in the topsoil where they degrade faster because microbial activity and soil temperature are greater. This is especially important for metsulfuron which is applied in spring and is apparently more susceptible to microbial degradation than chlorsulfuron.

Incidents of injury to following crops from autumn applications of chlorsulfuron are most likely in soils of high organic matter content or those which are imperfectly drained or at low-lying sites with a high water table. At these sites, the tapping of subsoil by a deep rooted and highly sensitive crop such as sugar beet and/or the return of the water to the topsoil by capillary action can lead to appreciable damage.

With the recent trend to autumn sowing of cereals, the possibility exists of similar incidents of damage to following crops with other acidic herbicides. The appearance of such chemicals in drainage water following leaching is also possible.

ACKNOWLEDGEMENTS

We thank Mr M May of the Norfolk Agricultural Research Station and Mr T Breay of British Sugar P.L.C. for kindly providing all the information on damage to sugar beet by chlorsulfuron.

REFERENCES

Anon (1983) 1:250,000 Map of the soils of England and Wales, Soil Survey of England and Wales, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, U.K.

Anderson, R.L., Humburg, N.E. (1987) Field duration of chlorsulfuron bioactivity in the Central Great Plains. *Journal of Environmental Quality* 16, 263-266.

Nicholls, P.H., Evans, A.A. (1985) Adsorption and movement in soils of chlorsulfuron and other weak acids. *Proceedings 1985 British Crop Protection Conference - Weeds* 1, 333-339.

Nicholls, P.H., Briggs, G.G. and Evans, A.A. (1983) Simulation of herbicide movement in soils in winter. *Aspects of Applied Biology* 4, 485-494.

Nicholls, P.H., Walker, A. and Baker, R.J. (1982) Measurement and simulation of the movement and degradation of atrazine and metribuzin in a fallow soil. *Pesticide Science* 13, 484-494.

Phillips, F.T. (1964) The aqueous transport of water-soluble nematicides through soils. III. *Journal of the Science of Food and Agriculture* 15, 458-463.

Richardson, W.G., West, T.M. and Parker, C. (1981) *Technical Report No. 62* Institute of Arable Crops Research, Weed Research Division, Long Ashton Research Station, Bristol, BS18 9AF. U.K. pp 44-52.

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

Walker, A.,, Brown, P.A. (1983) Measurement and prediction of chlorsulfuron persistence in soil. *Bulletin of Environmental Contamination and Toxicology* 30, 365-372.