Session 9A

Herbicide Safeners, Additives and Formulants

Chairman	Mr P B SUTTON
Session Organiser	Dr P J RYAN
Papers	9A-1 to 9A-3

HERBICIDE SAFENERS: RECENT ADVANCES AND BIOCHEMICAL ASPECTS OF THEIR MODE OF ACTION

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ABSTRACT

Herbicide safeners are a group of chemically diverse compounds with the ability to enhance crop tolerance to certain herbicides. Safeners have been developed for thiocarbamate, chloroacetanilide, sulfonylurea, and aryloxy-phenoxypropionate herbicides in major monocotyledonous crops including maize, small grain cereals, rice, and grain sorghum. There is considerable evidence that safeners act by accelerating herbicide metabolism and detoxification in these crops. Metabolic reactions that are enhanced include oxidation (hydroxylation, oxidative demethylation), glucose conjugation, and glutathione conjugation. A number of enzymes involved in these metabolic pathways, such as glutathione *S*-transferase isozymes and various forms of cytochrome P450-dependent monooxygenase activities, have been shown to be induced by safeners. Safeners have also been reported to increase the levels of glutathione and to enhance the activities of key enzymes of assimilatory sulfate reduction. The botanical specificity of safener protection will be discussed in the light of the recently developed aryloxyphenoxypropionate herbicide and safener combination, clodinafop-propargyl and cloquintocet-mexyl. The herbicide exhibits different metabolic routes in wheat (hydroxylation, ether cleavage, glycosidation) and target weeds (malate ester conjugation), and only crop-specific herbicide metabolism is enhanced by the safener.

INTRODUCTION

Weed management in modern agriculture requires efficient weed control technologies that are safe to the crop. The search for environmentally compatible herbicides with high biological activity and crop selectivity is, and will continue to be, a challenge for agrochemical research. During the last two decades several structurally diverse classes of herbicides with novel modes of action have been discovered and exploited for the commercial development of new crop selective weed control products. It can be expected that other biochemical targets and chemical lead structures with herbicidal potency will be discovered in the future by both 'random screening' and 'target site-directed' strategies (Bellus, 1991). However, insufficient crop tolerance is one of the major constraints in the development of new herbicides and in the use of existing herbicides in particular crops or under unfavourable environmental conditions. Based on the available knowledge of the mechanisms of herbicide selectivity, the chemical optimization of lead structures for crop selectivity will in the foreseeable future depend more on broad-based synthesis and testing of herbicidal molecules rather than on 'rational design' (Brown *et al.*, 1991). Recent efforts

are thus aimed at protecting crops from herbicidal injury by means of selection or genetic engineering of herbicide-tolerant crop cultivars (Hinchee *et al.*, 1993) Another approach that has been employed for approximately 25 years is to improve crop tolerance to new and existing herbicides by herbicide safeners.

THE SAFENER CONCEPT

Herbicide safeners are a group of chemically diverse compounds with the unique ability to protect crop plants from injury by certain herbicides without impairing weed control efficacy. The safener concept has been established and introduced into practical agriculture by the pioneering work of O. L. Hoffmann (Stephenson & Yaacoby, 1991). After the initial discoveries of naphthalic anhydride (naphthalene-1,8-dicarboxylic anhydride) and dichlormid (*N,N*-diallyl-2,2-dichloroacetamide) as herbicide safeners, a number of other safeners have been developed for all major monocotyledonous crop species including maize, small grain cereals, rice and grain sorghum. In most crop-weed associations, selective improvement of crop tolerance can be achieved with safeners applied as mixed formulations with the herbicide; however, seed-treatment with safeners is also currently used, mainly in grain sorghum, to protect only the crop and not botanically closely related weeds. Safeners have been exploited in two ways: to improve tolerance of newly developed herbicides with limited selectivity on target crops, and to extend the use of available herbicides on additional crops.

In the past, the search for safeners has been most successful for pre-plant soil incorporated and pre-emergence herbicides of the thiocarbamate and chloroacetanilide classes in maize, and for chloroacetanilides in sorghum and wet-sown rice. More recently, safeners have also been developed for post-emergence grass weed control in cereals in combination with aryloxyphenoxypropionate herbicides. Protection by various safeners has also been reported for members of several other herbicide classes such as the sulfonylureas, imidazolinones, cyclohexanediones, and isoxazolidinones. The history of the discovery of herbicide safeners, their biological performance and practical use is covered by recent reviews (Hatzios & Hoagland, 1989; Stephenson & Yaacoby, 1991).

MECHANISMS OF ACTION OF HERBICIDE SAFENERS

Despite extensive research efforts the protective mechanism of herbicide safeners is far from being completely understood. Several hypotheses have been advanced for the mechanism(s) of the protective action of herbicide safeners. It has been suggested that safeners may reduce herbicide uptake or translocation to sensitive site(s) within the plant, or increase the rate of herbicide metabolism and detoxification. Alternatively, safeners may prevent binding of the herbicide to the cellular target site, or antagonize herbicidal effects at the physiological level. Finally, a combination of several of these factors may be encountered. Understanding of herbicide-safener interactions is hampered by a general lack of exact knowledge of the herbicidal mechanisms ultimately leading to plant injury. However, there is a large body of evidence that the hitherto commercialized safeners protect crop plants by enhancing herbicide metabolism and detoxification. Possible mechanism(s) of action of safeners and experimental evidence for or against the various hypotheses have

been discussed in detail in several reviews (Hatzios & Hoagland, 1989; Hatzios, 1991) and will therefore not be addressed here. The present paper will emphasize some recent advances in the development of herbicide safeners and investigations of their biochemical mode of action.

Enhancement of herbicide metabolism

Differences in herbicide metabolism and detoxification between tolerant and susceptible plant species have long been recognized as one important mechanism contributing to the selective action of many herbicides (Owen, 1987). Biotransformations of herbicides in plants generally include oxidation, hydrolysis, reduction (rarely), and conjugation to *e.g.* glutathione, glucose, or amino acids. To date, two different metabolic pathways have been related to the mode of action of herbicide safeners. The first represents the conjugation of chloroacetanilide and sulfoxidized thiocarbamate herbicides with glutathione; the second includes hydroxylation and subsequent glucose conjugation. The latter pathway appears to be important predominantly in safener protection to aryloxyphenoxypropionate, sulfonylurea, and imidazolinone herbicides.

Glutathione conjugation and glutathione *S*-transferases

Conjugation of herbicides *via* the thiol function of reduced glutathione (γ-glutamyl-cysteinylglycine) is well established as one of the major detoxification and selectivity factors in plants (Lamoureux *et al.*, 1991). Though glutathione conjugations can proceed non-enzymatically at appreciable rates with some substrates, these reactions are usually accelerated through catalysis by glutathione *S*-transferase enzymes (EC 2.5.1.18). The rate of glutathione conjugation of herbicides in plants may be regulated, in principle, by both glutathione *S*-transferase level and activity as well as by glutathione availability.

Early studies have shown that safeners such as naphthalic anhydride and dichlormid enhance the rate of metabolism of the herbicide EPTC in maize, and Lay & Casida (1976) were the first to demonstrate that dichlormid elevated the levels of both glutathione and glutathione *S*-transferase activity towards EPTC-sulfoxide. The sulfoxidation of EPTC that precedes conjugation with glutathione to yield non-phytotoxic *S*-(*N*,*N*-dipropyl-carbamoyl)glutathione has a twofold effect: it is considered to be a bioactivation step yielding the phytotoxic EPTC-sulfoxide, and to enable subsequent spontaneous or enzymatically catalysed nucleophilic attack of glutathione. The relative importance of non-enzymatic and enzymatic glutathione conjugation of EPTC-sulfoxide is still a matter of debate. Sulfoxidation of EPTC has been ascribed to cytochrome P450-dependent monooxygenase activity; more recent evidence, however, indicates that sulfoxidation is mediated by a distinct microsomal haemoprotein, called peroxygenase, whose activity in maize was reported to be depressed in response to dichlormid-treatment (Blée, 1991).

Chloroacetanilide herbicides are initially metabolized in plants through glutathione conjugation by nucleophilic displacement of chlorine from the chloroacetyl side chain, without the need for a preceding metabolic step to increase electrophilicity. Fuerst & Gronwald (1986) have shown that protection of sorghum from metolachlor injury by oxabetrinil and other safeners is closely correlated with their ability to accelerate metolachlor metabolism in shoot tissue. Maize exhibits reduced tolerance to metolachlor and other chloroacetanilide herbicides under certain adverse growing conditions such as high

soil moisture and low soil temperatures before seedling emergence. Effects of soil temperature have been related in part to a slower rate of herbicide metabolism, as well as to greater herbicide exposure due to slower seedling emergence (Viger *et al.*, 1991). The recently developed safener, benoxacor, protects maize from metolachlor injury under a wide range of environmental conditions (Peek *et al.*, 1988). The predominant site of uptake of pre-emergence applied chloroacetanilides is the coleoptile of germinating grass species, while the primary anatomical sites affected are the enclosed developing leaves and apical and intercalary meristems (LeBaron *et al.*, 1988). After shoot application of metolachlor a comparatively small proportion of the herbicide moved into the enclosed developing leaves, and most of the absorbed herbicide was retained in the coleoptile and was metabolized there *via* glutathione conjugation (Kreuz *et al.*, 1989). The developing leaves, however, were found to be relatively slow to metabolize metolachlor as compared to the coleoptile. The developing leaves also exhibited the lowest glutathione *S*-transferase activity of all seedling tissues examined. Benoxacor significantly reduced the concentration of unmetabolized metolachlor mainly in the developing leaves, but also in the coleoptile and mesocotyl, as a consequence of enhanced metabolism. Activity of glutathione S-transferase accepting metolachlor as substrate was increased fivefold in seedling shoots upon benoxacor treatment (Kreuz *et al.*, 1989; Viger *et al.*, 1991). Similar results were reported from studies with metazachlor and the safener BAS 145138 (1-dichloroacetylhexahydro-3,3,8a-trimethyl-pyrrolo[1,2-*a*]-pyrimidin-6-(2*H*)-one) in maize (Fuerst & Lamoureux, 1992).

A number of studies have established the existence in plants of glutathione *S*-transferase families comprising several isozymes which exhibit varying degrees of substrate specificity for particular herbicides and non-herbicidal compounds. Some of these enzymes have been purified and characterized as soluble homo- or heterodimeric proteins with subunit molecular masses close to 25 kDalton each. In maize, sorghum and rice, herbicide safeners increase the extractable glutathione *S*-transferase activities as evaluated with the model substrate 1-chloro-2,4-dinitrobenzene, chloroacetanilide herbicides, and EPTC-sulfoxide (maize only). Constitutive and safener-induced glutathione *S*-transferases from sorghum and maize have been distinguished based on chromatographic elution characteristics and differential activities towards various substrates (Dean *et al.*, 1990; Fuerst *et al.*, 1993). Maize contains at least two major constitutive glutathione *S*-transferase isozymes accepting metolachlor as substrate and whose activities were enhanced by treatment of seedlings with benoxacor. A third such isozyme was found to be absent constitutively and highly induced by benoxacor (Fuerst *et al.*, 1993). Complementary DNAs for two maize glutathione *S*-transferases have been cloned and the deduced amino acid sequences were shown to possess some similarity to each other as well as to animal glutathione *S*-transferases (reviewed by Timmerman, 1989). Safener induction of glutathione *S*-transferase activity is associated with a net accumulation of enzyme protein and requires *de novo* protein synthesis. The steady-state levels of messenger RNA coding for a particular isozyme subunit were increased in maize treated with dichlormid or flurazole (benzyl 2-chloro-4-trifluoromethylthiazole-5-carboxylate), which suggests that regulation of enzyme synthesis by safeners is exerted at the level of gene transcription.

Regulation of glutathione levels and biosynthesis

Safeners have repeatedly been shown to increase the tissue concentration of reduced glutathione in plants, yet the significance of elevated glutathione levels for safener action is still uncertain. A weak correlation has been found between the increase in glutathione

content of sorghum shoots and the degree of protection from metolachlor injury conferred by a particular safener (Gronwald *et al.*, 1987). On the other hand, decreased glutathione contents of maize shoots due to treatment with buthionine sulfoximine, an inhibitor of γ-glutamylcysteine synthetase, are correlated with increased metolachlor susceptibility (Farago *et al.*, 1993). In roots of maize seedlings, dichlormid and benoxacor increased the contents of free cysteine and glutathione and enhanced the biosynthesis of these thiols from inorganic sulfate (Farago & Brunold, 1990). This was attributable to an increase in the extractable activities of adenosine 5'-phosphosulfate sulfotransferase and ATP-sulfurylase (EC 2.7.7.4), two key regulatory enzymes of assimilatory sulfate reduction. Glutathione reductase activity (EC 1.6.4.2) has also been reported to be enhanced in the shoots of safener-treated maize seedlings (Kömíves *et al.*, 1985). It has not been elucidated by which mechanism safeners interfere with the proposed negative feedback control exerted by cysteine and glutathione levels on the sulfate assimilation and thiol biosynthetic pathway.

Oxidative metabolism and cytochrome P450 monooxygenases

Oxidation and subsequent glucose conjugation constitutes a very important pathway responsible for herbicide selectivity that has recently also been associated with safener action. As will be discussed below, oxidation appears not always to afford complete herbicide detoxification, but is frequently a necessary and rate-limiting step for subsequent glucose conjugation. In recent years, safeners have been shown to stimulate oxidative metabolism of herbicides belonging to the groups of sulfonylureas, imidazolinones and aryloxyphenoxypropionates in plants *in vivo* (Hatzios, 1991).

There is accumulating evidence that cytochrome P450-dependent monooxygenases (EC 1.14.14.1) play a pivotal role in the oxidation of many herbicides in plants. The cytochromes P450 found in plants are, like the well-characterized ones in the endoplasmic reticulum from mammalian liver, membrane-bound haemoproteins of approximately 55 kDalton molecular mass (Donaldson & Luster, 1991). They require molecular oxygen for catalytic activity, NADPH and a second protein component, the flavoprotein NADPH-cytochrome P450 reductase. Reactions mediated by plant cytochrome P450 monooxygenases on herbicide substrates include aryl and alkyl hydroxylations and oxidative *N*- and *O*-demethylations. Conclusive evidence for the involvement of cytochrome P450 monooxygenases in plant herbicide metabolism has been obtained for 2,4-D, diclofop, bentazone, flumetsulam (*N*-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo[1,5-α]pyrimidine-2-sulfonamide), metolachlor and members of the phenylurea and sulfonylurea herbicide classes (Moreland *et al.*, 1993 and references cited therein; Frear *et al.*, 1993). The criteria that have been employed to demonstrate cytochrome P450 monooxygenase catalysis in those reactions include spectral evidence, photoreversible inhibition of these reactions by carbon monoxide, requirement for molecular oxygen and NADPH, sensitivity to known cytochrome P450 inhibitors and involvement of NADPH-cytochrome P450 reductase activity. Despite considerable experimental difficulties, *e.g.* low constitutive enzyme activity levels, apparent instability of the enzymes and presence of endogenous inhibitors in crude microsomal preparations, much effort has been devoted in recent years to elucidate the putative multiplicity of cytochrome P450 isoforms and their regulation implicated in safener action. These studies have revealed that microsomes isolated from plants treated with herbicide safeners or other xenobiotics, such as ethanol and phenobarbital, contain elevated cytochrome P450 monooxygenase activities towards particular substrates (Fonné-Pfister *et al.*, 1990; Frear *et al.*, 1991; Moreland *et al.*, 1993). Studies in grain sorghum

have shown that microsomal cytochrome P450-linked metabolism of bentazone (aryl hydroxylation), diazinon (desulfuration and oxidative dearylation) and lauric acid (in-chain hydroxylation) is stimulated to varying degrees by a number of safeners applied to the seed prior to planting (Moreland *et al.*, 1993). In contrast, cinnamic acid 4-hydroxylase, a well-known plant cytochrome P450 enzyme in the general phenylpropanoid pathway, was essentially unaffected, whereas oxidative *O*-demethylation of metolachlor (methoxypropyl side chain) was actually depressed. Cytochrome P450-mediated oxidation of several herbicides and its induction by naphthalic anhydride, ethanol, or phenobarbital has been demonstrated in wheat (Frear *et al.*, 1991). In this system, increases in monooxygenase activities as determined with diclofop, chlorsulfuron and triasulfuron as substrates ranged from five- to twentyfold, depending on the particular substrate and on the inducer employed. These and other results have provided indirect evidence that in plants multiple cytochrome P450 isoforms participate in the metabolism of the different herbicides and other substrates and furthermore that the various isoforms are differentially regulated by safeners. Interestingly, strong evidence has been obtained that in wheat diclofop aryl hydroxylase is identical with lauric acid (ω-1)-hydroxylase which, for the first time, links a herbicide-metabolizing monooxygenase activity to a particular cytochrome P450 isoform that participates in a defined physiological reaction (Zimmerlin & Durst, 1992). Both enzyme activities exhibited similar induction patterns with naphthalic anhydride and phenobarbital.

There is some indirect evidence that induction of cytochrome P450 isoforms by safeners, like the induction of glutathione *S*-transferases, might be exerted through enhanced gene expression. Safener-induction of sulfonylurea oxidation in maize leaves apparently requires *de novo* protein synthesis (Sweetser, 1985). However, herbicide-metabolizing cytochromes P450 have not yet been highly purified, neither have corresponding DNA sequences been determined to allow the analysis of their regulation by safeners at the protein or transcript level.

Glucose conjugation

Metabolism of herbicides to derivatives containing free hydroxy groups is generally followed by extensive carbohydrate conjugation, with *O*-β-D-glucosides representing the most common group of these conjugates (Lamoureux *et al.*, 1991). Glucose conjugation seems in some cases necessary to complete herbicide detoxification; since free hydroxylated metabolites do not commonly accumulate to significant levels in plants, glucosylation appears *prima facie* not an important site for safener action. During metabolism of chlorimuron-ethyl in maize, however, hydroxylation at the 5-position of the pyrimidine ring yielded the major metabolite, 5-hydroxychlorimuron-ethyl (Lamoureux & Rusness, 1992). Chlorimuron-ethyl causes injury to maize that can be alleviated by BAS 145138. This safener increased the capacity for 5-pyrimidyl-*O*-glucoside formation from chlorimuron-ethyl, and feeding experiments with 5-hydroxychlorimuron-ethyl revealed that the *in vivo* rate of glucosylation was indeed accelerated. The glucose conjugate was less inhibitory *in vitro* towards the target enzyme, acetolactate synthase, as compared to the free 5-hydroxychlorimuron-ethyl. Interestingly, the safener BAS 145138 increased, in addition to glucose conjugation, the capacity for pyrimidine-ring hydroxylation as well as glutathione conjugation of chlorimuron-ethyl in maize. As will be discussed below, evidence for a safener-induced increase in the rate of glycosylation has also been obtained with a hydroxylated aryloxyphenoxypropionate herbicide in wheat (Kreuz *et al.*, 1991).

The very few *O*-glucosyltransferases studied so far that accept herbicide derivatives as substrates are usually soluble enzymes utilizing uridine 5'-diphosphoglucose as the glucosyl donor. A constitutively expressed 6-hydroxybentazone glucosyltransferase was recently isolated from cultured soybean cells (Gallandt & Balke, 1993). The proposed induction of glucosyltransferases by safeners has apparently not yet been investigated.

Secondary metabolism of herbicide conjugates and compartmentation processes

Glutathione conjugates of herbicides in plants usually undergo extensive processing to *e.g.* cysteine or thiolactic acid derivatives and conjugates thereof with malonate, to name but a few (Lamoureux *et al.*, 1991). Simple glucose conjugates are frequently subject to secondary conjugations to carbohydrate or malonyl residues. Terminal products may be stored as soluble metabolites, presumably in the vacuole, or deposited as 'bound residues' into cell wall components. Formation of soluble secondary metabolites and bound residues from the initial glutathione conjugates of propachlor and metolachlor in maize was only marginally influenced by the safener BAS 145138 and therefore appeared to be of minor significance for safener action (Khalifa & Lamoureux, 1990).

Circumstantial evidence indicates a reduced mobility in the plant of herbicide conjugates as compared to the parent herbicides (see *e.g.* Fuerst & Lamoureux, 1992). This is conceivably due to the lower membrane permeability of hydrophilic conjugates, but specific compartmentation processes have also been inferred. The commonly observed addition of a malonyl residue to initially formed glucose conjugates or glutathione-derived cysteine and thiolactic acid conjugates has been proposed to be a mechanism to facilitate transport into the vacuole (Lamoureux *et al.*, 1991). However, vacuolar localization of herbicide conjugates has rarely been demonstrated, and transport processes have apparently not been investigated as yet. Only recently, active transport of the glutathione conjugates of metolachlor and other xenobiotics into the plant vacuole has been discovered and shown to be mediated by an ATP-dependent carrier in the tonoplast membrane (Martinoia *et al.*, 1993). This vacuolar carrier showed a striking resemblance to the glutathione *S*-conjugate export pump in the canalicular membrane of mammalian liver. It is not yet known whether such transport processes into plant vacuoles are influenced by herbicide safeners.

Specificity of safener action

Safeners used as a tank-mixture or prepackaged formulation with the herbicide act specifically with respect to the plant species that are protected from herbicidal injury. Fenchlorazole-ethyl, a compound developed for use in conjunction with the aryl-oxyphenoxypropionate herbicide, fenoxaprop-ethyl, has been reported to act as both a safener on wheat and as a synergist of herbicidal action on *Digitaria ischaemum* (Yaacoby *et al.*, 1991). In both plant species, fenchlorazole-ethyl stimulated deesterification of fenoxaprop-ethyl to the herbicidally active free acid. Further metabolism and detoxification of the herbicide, however, was only enhanced in wheat but not in *D. ischaemum*. More recently, specific safener action of cloquintocet-mexyl (5-chloro-8-quinolinoxyacetic acid-1-methylhexyl ester) for the herbicide clodinafop-propargyl (2-propynyl-*R*-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]propionate) on wheat (Amrein *et al.*, 1989) could be explained on the basis of the qualitatively different metabolic pathways of the herbicide in wheat and in susceptible target weeds. Metabolism of clodinafop-propargyl in wheat

proceeded through deesterification to the herbicidally active acid, followed by hydroxylation and ether cleavage to yield 2-[4-(6-hydroxy-5-chloro-3-fluoro-2-pyridinyloxy)phenoxy]-propionic acid and 2-(4-hydroxyphenoxy)propionic acid, respectively. All metabolites were subject to carbohydrate conjugation. In excised wheat leaves, rapid deesterification occurred, while all subsequent metabolic steps where significantly enhanced by the safener cloquintocet-mexyl (Kreuz *et al.*, 1991). In leaves of *Alopecurus myosuroides* and *Lolium rigidum*, the readily formed free acid of clodinafop-propargyl was slowly converted to a major metabolite that was identified by mass spectrometry, ^{1}H-nmr and chemical synthesis as the ester conjugate of the herbicide acid with malate (Kreuz *et al.*, unpublished results). No oxidative metabolism of clodinafop-propargyl was detected in these weed species. The rates of deesterification of clodinafop-propargyl and re-esterification with malate were not influenced in these weeds by the safener cloquintocet-mexyl. Thus, the metabolic pathway conferring moderate herbicide tolerance to wheat in the absence of cloquintocet-mexyl and which is enhanced by the safener to confer full tolerance is completely absent in these susceptible weeds.

CONCLUDING REMARKS

Herbicide safeners act at multiple sites of herbicide metabolism and detoxification pathways in plants by enhancing oxidative reactions, glucose conjugation, glutathione conjugation, and glutathione biosynthesis. There are indications that induction of metabolism is exerted at the level of transcription of genes coding for herbicide-metabolizing enzymes. Safeners appear to enhance, in a particular plant species, metabolic pathways that are already expressed at a certain constitutive level, rather than to induce qualitatively different reactions. The molecular mechanisms involved in these induction processes, however, still remain elusive and require further research.

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MODE OF ACTION OF NAPHTHALIC ANHYDRIDE AS A MAIZE SAFENER FOR THIFENSULFURON-METHYL

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ABSTRACT

Thifensulfuron-methyl applied preplant incorporated (ppi) at 32, 64, and 96 g/ha caused significant injury to 'Northrup King 9283,' 'Garst 8532,' and 'Pioneer 3377' hybrids of maize. Naphthalic anhydride (NA) used as seed dressing at 0.5% (wt/wt) increased the tolerance of all maize hybrids to ppi-applied thifensulfuron-methyl. When the herbicide was applied early postemergence (post-em.), injury to maize hybrids occurred only at the high rates of 64 and 96 g/ha. NA did not protect any maize hybrid against post-em.-applied thifensulfuron. Metabolism studies with cut coleoptiles of all maize hybrids showed that NA enhanced the deesterification of thifensulfuron-methyl causing a 30-50% increase in the formation of the parent acid, thifensulfuron. These results suggest that NA may protect maize against thifensulfuron-methyl by inducing the activity of hydrolytic enzymes such as carboxylesterases.

INTRODUCTION

Thifensulfuron-methyl, designated previously as DPX-M6316 or thiameturon, is a selective sulfonylurea herbicide used for broadleaved weed control in cereal grains and soybean (Long et al., 1988; Sionis et al., 1985).

The observed selectivity of thifensulfuron-methyl in wheat and soybean appears to result from the rapid metabolic inactivation of this herbicide (Brown et al., 1990; Cotterman & Saari, 1989). Rapid deesterification of thifensulfuron-methyl to its inactive free acid was found to be the major metabolic reaction contributing to the tolerance of soybean and wheat (Brown et al., 1990; Cotterman & Saari, 1989). Sensitive weeds were less efficient in metabolizing this herbicide.

Maize is sensitive to thifensulfuron-methyl as well as to several other sulfonylurea herbicides (Brown, 1990; Eberlein & Miller, 1989). Eberlein et al. (1989) evaluated the response of several maize genotypes to thifensulfuron-methyl. They found that differential metabolism was also responsible for the observed differential tolerance of maize genotypes to this herbicide, but the identity of the detected metabolites of thifensulfuron-methyl was not revealed.

The sensitivity of maize genotypes to sulfonylurea herbicides can be greatly improved with the use of safeners such as naphthalic anhydride (NA) or dichloroacetamide derivatives (Barrett, 1989; Devlin & Zbiec, 1991; Hatzios, 1984; Lamoureux & Rusness, 1991; Sweetser, 1985). The pro-

tection of maize by NA and other safeners against chlorsulfuron and chlorimuron-ethyl injury has been linked to a safener-induced enhancement of herbicide metabolism (Lamoureux & Rusness, 1991; Sweetser, 1985).

The objectives of the present study were to a) evaluate the efficacy of NA as a safener of three maize hybrids against injury caused from ppi and early post-em. applications of thifensulfuron-methyl and b) determine the potential effects of NA on the uptake and metabolism of ^{14}C-thifensulfuron-methyl by excised coleoptiles of etiolated seedlings of these three hybrids of maize.

MATERIALS AND METHODS

Interactions of NA and thifensulfuron-methyl on maize

The maize hybrids used in the present study were 'Northrup King 9283,' 'Garst 8532' and 'Pioneer 3377.' The safener NA was applied as a seed dressing at 0.5% (wt/wt). Safened and unsafened seeds of the three maize lines were grown, three plants per 476 ml styrofoam cup, in a potting medium of weblite, vermiculite, and sphagnum peat moss (2:2:1, V/V/V) supplemented with a controlled-release fertilizer. Thifensulfuron-methyl, formulated as 75% dry flowable, was applied either ppi or early post-em. at 0, 32, 64, and 96 g/ha with a CO_2-pressurized backpack sprayer delivering 190 l/ha spray mix at 220 kPa pressure with even flat spray tips. A nonionic surfactant (X-77) was included at 0.125% of the spray volume at the early post-em. application of the herbicide, which was conducted when maize seedlings were 10-cm tall. After treatment with the herbicide and planting of the NA-safened and unsafened seeds, the cups were placed in a greenhouse with a 14 h photoperiod and 22/29 ^{o}C night/day temperature.

The experiment was designed as a 4 x 2 (herbicide x safener) factorial with two replications and three subreplications and was repeated in time. Three weeks after the ppi treatment and two weeks after the early post-em. treatment with the herbicide, maize shoots were harvested, dried in a forced-air oven at 50 ^{o}C and weighed. Standard errors of observed mean responses were calculated and data are presented as histograms comparing unsafened and NA-safened maize seedlings at each rate of thifensulfuron-methyl.

Effect of NA on thifensulfuron-methyl uptake and metabolism in maize

NA-safened and unsafened seeds of the three maize hybrids were germinated on wet paper towels in a dark incubator for 3 days at 30 ^{o}C. The coleoptiles of the etiolated maize seedlings were cut to 2-cm long pieces, which were incubated for 48 h into 2 ml of an aqueous solution containing 27 nCi (600,000 dpm) of (triazine 2-^{14}C)-labeled thifensulfuron-methyl. The treated coleoptiles were then washed with 80% methanol for 1 min and ground in a mortar and pestle with 1 ml of 80% acetone. This process was repeated three times and the recovered radioactivity in the combined extracts of maize coleoptiles was determined by liquid scintillation counting (LSC). The extracts were then concentrated under an air stream and analyzed by thin-layer chromatography (tlc) using a solvent system containing acetonitrile and water (75:25, V/V). The metabolites of thifensulfuron-methyl were detected by x-ray autoradiography and radioactivity in each spot was determined by LSC after scraping of the tlc plates.

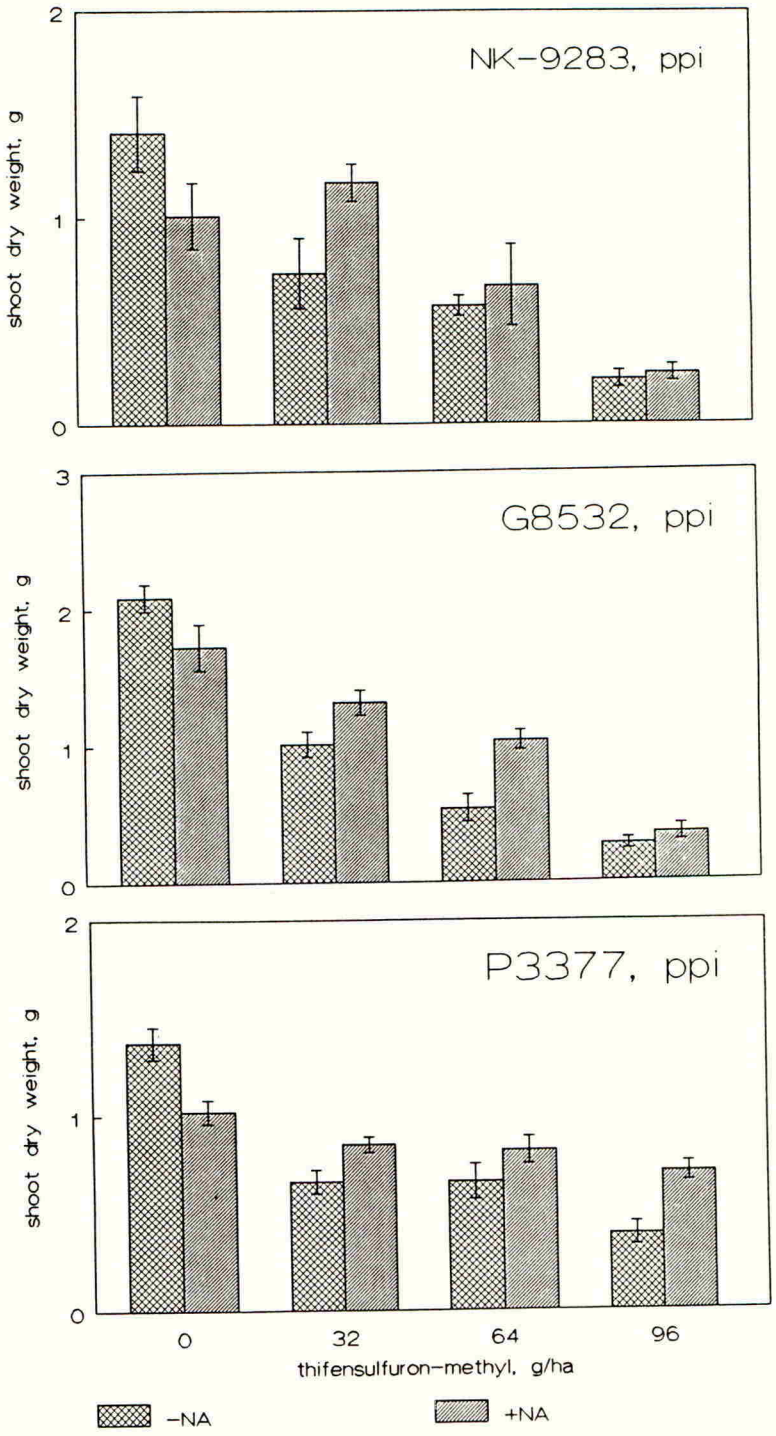

FIGURE 1. Protection of three maize hybrids from thifensulfuron-methyl (ppi-applied) injury by seed treatment with the safener NA.

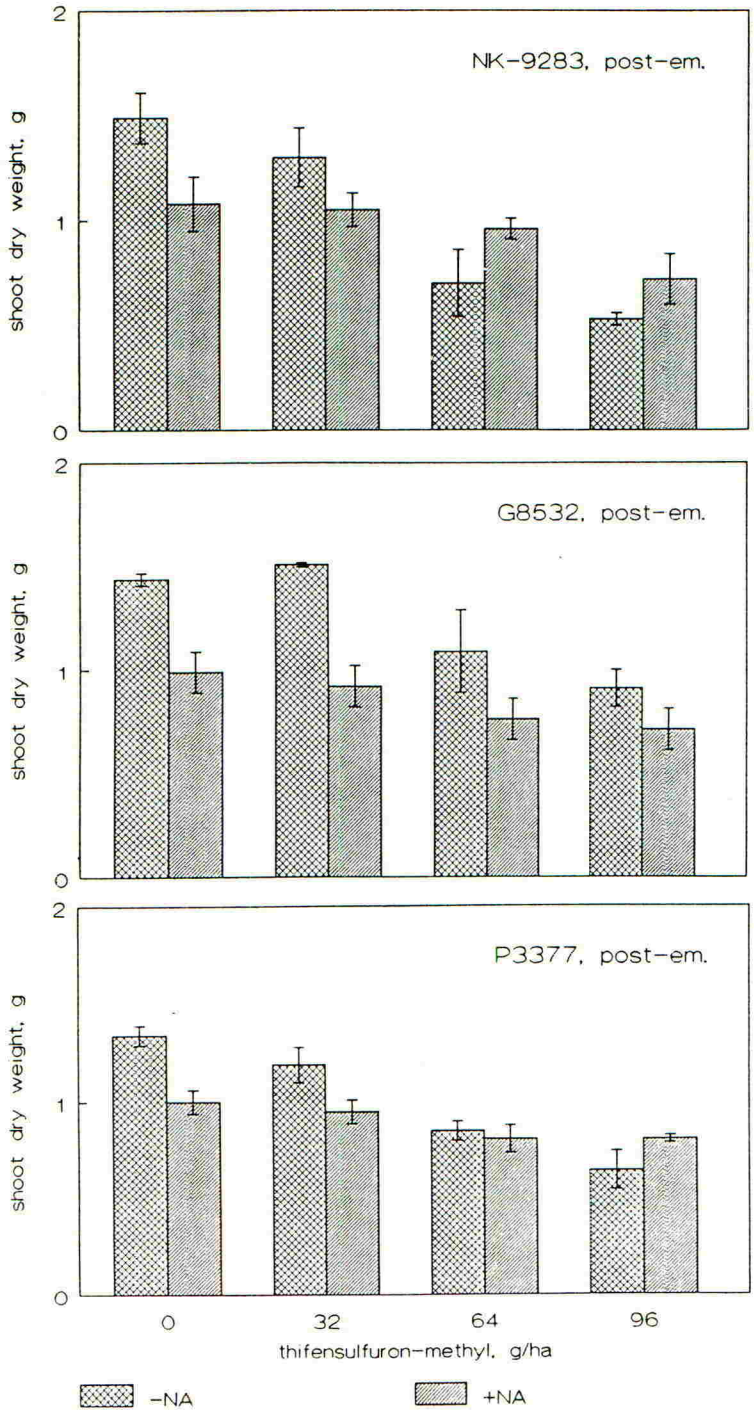

FIGURE 2. Protection of three maize hybrids from thifensulfuron-methyl (post-em.-applied) injury by seed treatment with the safener NA.

Analytical samples of thifensufluron-methyl and thifensulfuron acid were co-chromatographed with the maize extracts on the tlc plates.

RESULTS AND DISCUSSION

Interactions of NA and thifensulfuron-methyl on maize

Thifensufluron-methyl applied ppi at 32, 64, and 96 g/ha caused significant injury to 'Northrup King-9283,' Garst 8532' and 'Pioneer 3377' hybrids of maize (Figure 1). Seed treatment with NA applied at 0.5% (wt/wt) was moderately effective in protecting the three maize genotypes against injury caused by 32 and 64 g/ha, but not by 96 g/ha, of ppi-applied thifensulfuron-methyl. Following early post-em. applications, thifensulfuron-methyl reduced the growth of the aforementioned hybrids of maize only at the high rates of 64 and 96 g/ha (Figure 2). NA offered very little or no protection to any maize hybrid against injury caused by post-em.-applied thifensulfuron-methyl. NA applied alone caused a slight injury to seedlings of all maize hybrids (Figures 1 and 2). Nevertheless, this NA-induced injury is transitory and does not have any adverse effects on the yield of safened maize plants (Hatzios, 1984).

Effects of NA on thifensulfuron-methyl metabolism and uptake

Data in Figure 3 show that after 48-h of incubation, maize coleoptiles absorbed about 15-20% of the applied radioactivity. The levels of absorbed radioactivity by NA-safened and unsafened coleoptiles of the 'Garst 8532' and 'Pioneer 3377' hybrids of maize were very similar (Figure 3). NA reduced slightly the amount of radioactivity absorbed by the coleoptiles of 'Northrup King-9283' maize, but this effect was not statistically significant. Thus, NA does not appear to protect any of the three maize hybrids by reducing the uptake of thifensulfuron-methyl.

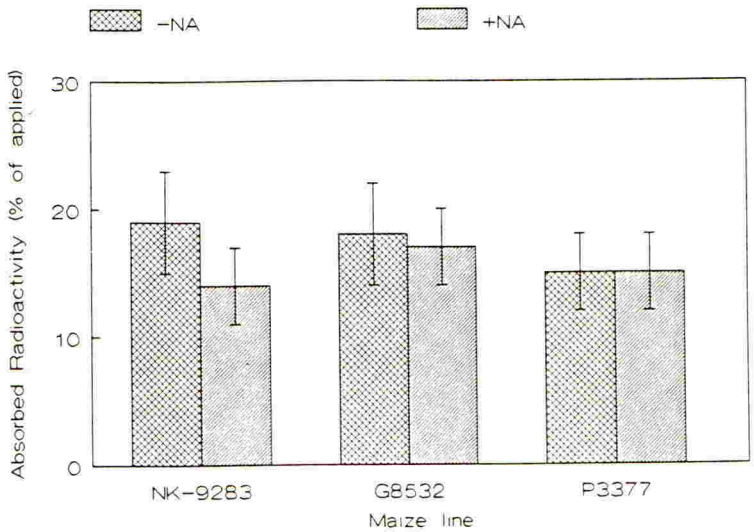

FIGURE 3. Absorption of ^{14}C-thifensulfuron-methyl by coleoptiles excised from unsafened and NA-safened seedlings of three maize hybrids.

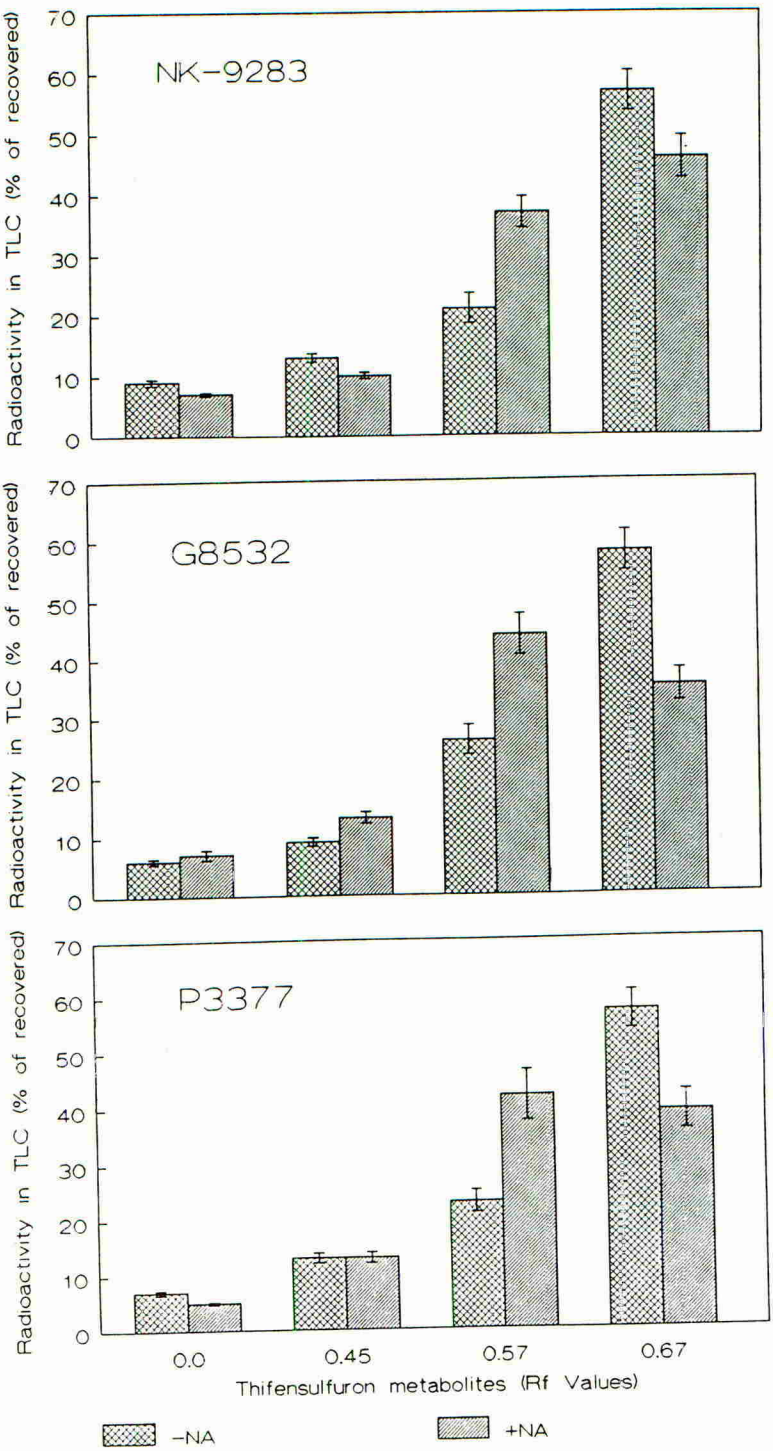

FIGURE 4. Metabolite profiles of thifensulfuron-methyl, detected by tlc analysis of coleoptile extracts from NA-safened and unsafened seedlings of 'Northrup King 9283,' 'Garst 8532,' and 'Pioneer 3377' maize.

Analysis of coleoptile extracts of NA-safened and unsafened seedlings of the three maize hybrids by tlc revealed the detection of four radioactive spots (Figure 4). The spot with Rf value of 0.67 corresponds to unmetabolized herbicide since it migrated to the same distance as the radiolabeled standard of thifensulfuron-methyl. The major metabolite detected had an Rf value of 0.57 and migrated to the same distance as the analytical standard of the deesterfied thifensulfuron acid. Two other minor metabolites (Rf values of 0.0 and 0.45) were also detected, but their identity is currently unknown. Treatment with NA enhanced significantly the rate of the deesterification reaction of thifensulfuron-methyl causing a 30-50% increase in the formation of thifensulfuron acid and a concomitant decrease in the levels of the parent methyl ester of this herbicide (Figure 4).

The obtained results support the hypothesis that the mode of action of NA in safening maize against injury from thifensulfuron-methyl is due to a stimulation of the rate of deesterification of this herbicidal ester to the inactive thifensulfuron acid. This deesterification reaction is believed to be catalyzed by a hydrolytic enzyme such as carboxylesterase, which has not been characterized as yet. Thus, in addition to glutathione S-transferases (GSTs), cytochrome P450-mediated monooxygenases, and UDP-glucosyl transferases (UDPGTs) (Hatzios, 1991), hydrolytic enzymes may also be enhanced by herbicide safeners that are known to protect grass crops against herbicide injury. The enzymatic properties of the thifensulfuron-methyl carboxylesterase from maize are currently under study.

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SELECTIVE GRASS-WEED CONTROL IN WHEAT AND BARLEY BASED ON THE SAFENER FENCHLORAZOLE-ETHYL

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ABSTRACT

Trials carried out throughout England and Scotland with the safener fenchlorazole-ethyl formulated with fenoxaprop-p-ethyl (Hoe 6001) for wheat and fenoxaprop-p-ethyl plus diclofop-methyl (Hoe 1825) for barley, showed high and consistent levels of control of *Alopecurus myosuroides, Avena spp.* and other annual grasses. Hoe 6001 applied at 1.0, 1.25 and 1.5 l/ha in autumn/winter, early and late spring, respectively, gave a mean of 97% control of *A. myosuroides* and 99% control of *Avena spp.* in wheat. In barley Hoe 1825 at 2 l/ha in the autumn/winter or 2.5 l/ha in early spring gave 97% control of *A. myosuroides* and *Avena spp.* Crop tolerance trials show good selectivity to Hoe 6001 in wheat and Hoe 1825 in barley on all the cultivars and at all the growth stages tested.

INTRODUCTION

Fenoxaprop-ethyl or the active isomer fenoxaprop-p-ethyl are highly effective foliar-applied graminicides but the tolerance of wheat and barley is marginal. Selective use of these products in cereals was made possible following the discovery by Hoechst AG of the safener fenchlorazole-ethyl (Hoe 70542) (Bieringer *et al*, 1989), which is applied with the active ingredient in a novel oil-in-water emulsion formulation. Hoe 70542 has no herbicidal activity, and works by accelerating the metabolic breakdown of fenoxaprop to non-phytotoxic degradation products in wheat and barley (Köcher *et al.*, 1989). This without any apparent effect on the herbicidal activity of fenoxaprop in the target weeds.

The development of formulations of fenchlorazole-ethyl with fenoxaprop-p-ethyl (Hoe 6001, 'Cheetah Super', Hoechst) for *Alopecurus myosuroides* and *Avena spp.* control in wheat, and with fenoxaprop-p-ethyl and diclofop-methyl (Hoe 1825, 'Tigress', Hoechst) for barley, is described in this paper.

MATERIALS AND METHODS

All trials were carried out on commercially-grown crops of winter and spring wheat or barley in England and Scotland covering a range of locations and cultivars. Treatments were applied at the appropriate rate for each of three growth stages *(Lawson & Read,* 1992) viz. up to GS 23, 30 or 39 using Van der Weij 'AZO' precision sprayers at an operating pressure of 250 k Pa, delivering 200-300 l/ha using eight flat fan nozzles spaced 25 cm apart on a 2 m boom.

On wheat one hundred and forty-seven efficacy trials were carried out between 1985 to 1992 on *A. myosuroides* and one hundred and twenty-three on *Avena spp.* In addition, twenty seven trials were carried out on other grass weeds, including *Poa trivialis, Apera spica-venti, Phalaris paradoxa* and *Phleum pratense*. On barley a total of thirty six efficacy trials were carried out between 1988 and 1992 on *A. myosuroides* and ninety on *Avena spp.* In addition, there were six trials on *Lolium spp.*

A further one hundred and eleven trials were carried out on weed-free sites between Autumn 1985 and Spring 1992. Single and double doses of Hoe 6001 and Hoe 1825 were applied to a range of winter and spring wheat or barley varieties, at a range of growth stages (Zadoks *et al.*,1974) to investigate crop tolerance. The trial layout consisted of randomised blocks replicated four times.

Herbicides used in the trials and their active ingredient content were fenoxaprop-p-ethyl, 55 g/l + fenchlorazole-ethyl, 'Hoe 6001' oil in water emulsion; diclofop-methyl, 'Hoegrass'(Hoechst), 380 g/l EC; fenoxaprop-p-ethyl + diclofop-methyl 14 g/l + 313 g/l + fenchlorazole-ethyl, 'Hoe 1825' oil in water emulsion; difenzoquat, 'Avenge 2'(American Cyanamid Co.), 15% w/v SC; flamprop-M-isopropyl, 'Commando'(Shell), 200 g/l EC; isoproturon, 'Arelon'(Hoechst) 553 g/l SC.

Counts of grass-weed seed heads were carried out as soon as all had emerged, using random quadrats which varied in size from 0.1 to 0.5 m^2 according to the density of the weed. In the tolerance trials, crop vigour was assessed at one week, three weeks and monthly intervals following application, using a percentage scale. Yields from all the tolerance trials and some efficacy trials were taken using Hege small plot combines and corrected to 15% moisture content.

RESULTS

Wheat

Hoe 6001 gave a mean of 97% control of *A. myosuroides* (Table 1) and 99% control of *Avena spp.* (Table 2). A mean of 95% control of *P. trivialis P. pratense, P. paradoxa,* and *A. spica-venti* was obtained with Hoe 6001 up to GS39 of the weeds.

In the crop tolerance trials no effects on crop vigour were observed, nor were there any significant reductions in yield (Table 3).

Barley

A mean of 97% control of *A. myosuroides* in winter barley was obtained with Hoe 1825 (Table 4), and a mean of 97% control of *Avena spp.* in winter and spring barley (Table 5). A mean of 95% control of *Lolium spp.* up to GS 30 was achieved with the appropriate rate of Hoe 1825.

In absence-of-weed trials no persistent crop vigour effects were noted, nor were there any significant reductions in crop yield (Table 6).

TABLE 1. Percentage control of *Alopecurus myosuroides* in wheat from applications made at three weed growth stage timings

		Mean (range)		
Treatment	Rate (l/ha)	up to GS 23	up to GS 30	up to GS 39
Hoe 6001	1.0,1.25,1.5	98 (94-100)	98 (87-100)	95 (85-100)
isoproturon	4.5,3.75, -	79 (23-100)	69 (0-98)	
flamprop-M-isopropyl	- , - , 3.0			72 (37-100)
Untreated (heads/m²)		940	910	660
Number of trials		42	69	36

TABLE 2. Percentage control of *Avena spp.* in wheat from applications made at three weed growth stage timings

		Mean (range)		
Treatment	Rate (l/ha)	up to GS 23	up to GS 30	up to GS 39
Hoe 6001	1.0,1.25,1.5	99 (94-100)	99 (96-100)	98 (81-100)
diclofop-methyl	1.5, - -	94 (64-100)		
difenzoquat	- , 6.6 -		89 (26-100)	
flamprop-M-isopropyl	- , - , 3.0			86 (20-100)
Untreated (heads/m²)		81	75	91
Number of trials		40	38	45

DISCUSSION

The level of control of *A. myosuroides* and *Avena spp.* achieved with Hoe 6001 (wheat) and Hoe 1825 (barley) was extremely high, irrespective of the time of application. Control was consistent from site to site, and season to season unlike standard treatments which tended to give more variable levels of control due to various soil and/or weather conditions. These results are consistent with those reported for the racemate fenoxaprop-ethyl (Read & Hewson 1990; Palmer & Read 1991) and for the mixture with diclofop-methyl (Hoe 1825) in Scotland (D'Souza *et al,* 1993).

Crop safety and yield data confirmed that fenchlorazole-ethyl formulated with fenoxaprop-p-ethyl alone or together with diclofop-methyl is very safe to the crop at all timings up to GS39. This demonstrates the efficacy of fenchlorazole-ethyl as a safener for both wheat and barley.

The consistent and high levels of grass weed control coupled with good crop safety and the wide window of application of Hoe 6001, and Hoe 1825, formulated with the safener fenchlorazole-ethyl allows farmers to benefit from improved levels of grass weed control in winter and spring sown wheat and barley.

TABLE 3. Relative wheat yields (untreated = 100) from applications made at three crop growth stages

Treatment	Rate (l/ha)	Mean (range) up to GS 23	up to GS 30	up to GS 39
Hoe 6001	1.0, 1.25, 1.5	99 (94-106)	99 (97-100)	100 (96-100)
	2.0, 2.5, 3.0	101 (94-109)	101 (98-105)	99 (97-100)
Standard	x 1	100 (92-104)	95 (88-105)	98 (95-101)
	x 2	101 (94-107)	99 (93-104)	97 (88-106)
Untreated (t/ha)		7.9	8.0	7.8
Number of trials		7	13	12

Standard up to GS 23 diclofop-methyl at 3.0 and 6.0 l/ha.
Standard up to GS 30 difenzoquat at 6.6 and 13.2 l/ha.
Standard up to GS 39 flamprop-M-isopropyl at 3.0 and 6.0 l/ha.

TABLE 4. Percentage control of *Alopecurous myosuroides* in winter barley from applications made at two different weed growth stages.

Treatment	Rate (l/ha)	Mean (range) up to GS 23	up to GS 30
Hoe 1825	2.0	98 (94-100)	-
	2.5	-	96 (81-100)
isoproturon	4.5	81 (16-100)	-
	3.75		87 (53-100)
Untreated (heads/m²)		420	330
Number of trials		18	18

TABLE 5. Percentage control of *Avena spp* in winter and spring barley from applications made at different weed growth stages.

Treatment	Rate (l/ha)	Mean (range)			
		Winter barley		Spring barley	
		up to GS 23	up to GS 30	up to GS 23	up to GS 30
Hoe 1825	2.0	98 (84-100)		94 (89-100)	
	2.5		98 (88-100)		93 (85-100)
diclofop-methyl	1.5	94 (57-100)		86 (70-100)	
difenzoquat	6.6		90 (42-100)		
flamprop-M-isopropyl	3.0				92 (82-100)
Untreated (heads/m²)		71	100	120	140
Number of trials		42	18	16	14

TABLE 6. Relative winter and spring barley yields (untreated = 100) from applications made at two crop growth stages.

Treatment	Rate (l/ha)	Mean (range)			
		Winter barley		Spring barley	
		up to GS 23	up to GS 30	up to GS 23	up to GS 30
Hoe 1825	2.5	97 (96-100)	96 (94-106)	97 (95-103)	98 (97-100)
	5.0	98 (96-100)	98 (97-100)	97 (93-100)	99 (94-102)
diclofop-methyl	2.5	96 (91-109)		95 (86-101)	
	5.0	95 (93-104)		91 (69-98)	
flamprop-M-isopropyl	3.0		97 (88-106)		97 (93-103)
	6.0		98 (95-101)		94 (79-104)
Untreated (t/ha)		6.6	6.9	5.3	5.5
Number of trials		12	12	17	14

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Session 9B

Fate and Significance of Herbicide Residues in Soil and Water - Measurement

Chairman:	Dr A D CARTER
Session Organiser	Dr A WALKER
Papers	9B-1 to 9B-4

ROLE OF FIELD STUDIES IN ASSESSING ENVIRONMENTAL BEHAVIOUR OF HERBICIDES

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ABSTRACT

Field studies, in conjunction with laboratory studies and computer modelling, can be important tools in understanding the behaviour of agricultural chemicals in the environment. Degradation rates measured in field dissipation studies can be used in computer models to estimate the environmental fate for a range of soils and weather conditions. Field studies can also be used to determine important transport mechanisms under field conditions and, when necessary, to develop management practices for reducing such transport mechanisms. Regulatory requirements for field studies should be flexible to allow tailoring sampling protocols to the objectives of the study, site conditions, and the properties of the agricultural chemical under study.

INTRODUCTION

The behaviour of agricultural chemicals in the environment is complex and influenced by many factors. Therefore, during the registration process a variety of studies must be performed to provide information on the behaviour of agricultural chemicals in soil and water. These studies include laboratory studies, lysimeter studies, and field studies. As a result of the EEC directive on Uniform Principles, the European registration requirements for field studies are being expanded. Non-routine studies are increasingly being requested by registration authorities. The purpose of this paper is to review the role of field studies in assessing the behaviour of agricultural chemicals in the environment. The design of such studies and sampling techniques will not be discussed since other papers provide the author's views on these topics (Jones and Norris, 1991; Norris *et al.*, 1991; Kirkland *et al.*, 1991).

ROLE OF FIELD STUDIES

Study Characteristics

An understanding of the nature of laboratory, lysimeter, and field studies is necessary for determining the role of field studies. Some of the characteristics of the different studies are summarised in Table 1.

One of the main distinguishing characteristics of field studies is that radiolabelled compounds cannot be used (except in very small-scale studies). Therefore, relevant metabolites must be identified and analytical methodology must be available prior to conducting any field study.

TABLE 1. Characteristics of typical laboratory, lysimeter, and field studies.

study characteristic	laboratory	lysimeter	field
radiolabelled material	yes	yes	no
soil structure maintained	no	yes	yes
soil sampling during experiment	yes	no	yes
simulates actual conditions	no	yes	yes
material balance	yes	yes	no
behaviour in subsoils	no	no	yes

One of the advantages of field studies is that the soil structure is maintained, while most laboratory studies are conducted with disturbed soil. Lysimeter studies are often conducted with intact cores, which preserve local structure but may not be representative of field scale characteristics such as intermittent lenses and perched water tables.

Collection of soil samples can be performed at regular intervals in field studies without affecting study results, but in lysimeter studies soil sampling is now not recommended except at the end of the study. In studies using small diameter lysimeters, this limitation is overcome by making applications to a number of lysimeters. At specified time intervals individual lysimeters are removed and sampled. A similar approach is often used in laboratory experiments.

Field studies are usually the best measure of degradation rates under actual use conditions. Lysimeter studies can closely approximate field conditions. Usually, laboratory experiments are conducted under carefully controlled conditions such as constant temperature, so that results are reproducible and results from different experiments can be more easily compared. Field studies are usually necessary for the study of field scale processes such as runoff and drainage, although laboratory and lysimeter studies may be useful in understanding such processes.

One of the disadvantages of field studies is the difficulty in obtaining a good material balance. The use of radiolabelled material in laboratory and lysimeter studies facilitates such a balance. However, in field studies, measurements can only be made of the parent and relevant metabolites, so that the nature of the degradates cannot be determined. For example, losses due to bound residues or mineralisation are not distinguishable in field studies. Another problem encountered in field studies is the variability of samples. For example, a coefficient of variation of about 110 percent is common, so 15-20 cores are required to achieve a 95% confidence interval of only ± 50 percent (Jones *et al.*, 1986). Therefore, field studies must be appropriately designed to meet study objectives and to utilise sampling and analytical resources efficiently.

Another advantage of field studies is that the behaviour of a compound can be studied in undisturbed subsoils and groundwater. Degradation below the root zone can be an important process for some compounds that have the potential to move below the root zone.

Usefulness of Laboratory, Lysimeter, and Field Studies

Because of the different nature of laboratory, lysimeter, and field studies, each type of study has an important role in providing information on the behaviour of an agricultural chemical in soil and water. A variety of standard tests have been developed for registration requirements. In addition, appropriate studies or combinations of studies sometimes must be developed to provide additional information.

Information on degradation mechanisms including microbial degradation, hydrolysis, and photolysis is usually best determined in laboratory experiments under controlled conditions. The ability to use radiolabelled compounds facilitates the determination of the degradation pathway and identification of any metabolites that may be formed. The mobility of parent compound and metabolites are usually best characterised by laboratory sorption (K_{OC}) measurements.

Large diameter lysimeter studies are being increasingly used to study the potential for an agricultural chemical to move through the upper metre of soil (Fuhr *et al.*, 1991; Fuhr and Hance, 1992). Since lysimeter cores can be placed outdoors and crops grown on the surface, actual use conditions can be approximated. The ability to use radiolabelled compounds allows the detection of very small amounts of compounds that may be present in the leachate exiting the bottom of the lysimeter. Small diameter lysimeter studies can be useful in obtaining preliminary information on promising compounds early in development. As soon as radiolabelled material is available, applications can be made to several lysimeters. At appropriate

intervals, individual lysimeters are removed and sampled to determine which metabolites are formed under field conditions as well as to provide information on the persistence and mobility of parent and these metabolites.

Determination of degradation and transport under field conditions is usually best accomplished through field studies such as field dissipation studies. Field studies can be used to measure behaviour in surface and subsoils and ground and surface water. Degradation rates measured in field studies can then be used in models along with laboratory Koc measurements to predict behaviour under other conditions. Because field studies usually entail the collection and analysis of a large number of samples, sampling schedules should be sufficiently flexible to allow for differences in study objectives, chemical properties, and site conditions. When possible, analyses should be performed as the study progresses so that results can be used to guide activities at later sampling intervals.

Field studies can also be used to identify mechanisms for movement of agricultural chemicals in soil and water under field conditions. Examples of such mechanisms include runoff, leaching, preferential flow, and drainage. Once the important transport mechanisms have been identified for a particular situation, field studies can be used to develop/demonstrate effective management practices for reducing the movement of agricultural chemicals via these mechanisms.

EXAMPLES

The use of field studies in the manner described in the previous section can be illustrated with two recent studies conducted with a cereal herbicide. The first study, conducted in the United Kingdom, shows how field studies can be used to determine degradation rates in soil as well as to determine the most important mechanisms resulting in residues in surface water. The second study, conducted in Germany, illustrates how field studies can be used in the development of management practices to reduce movement of agricultural chemicals into surface water.

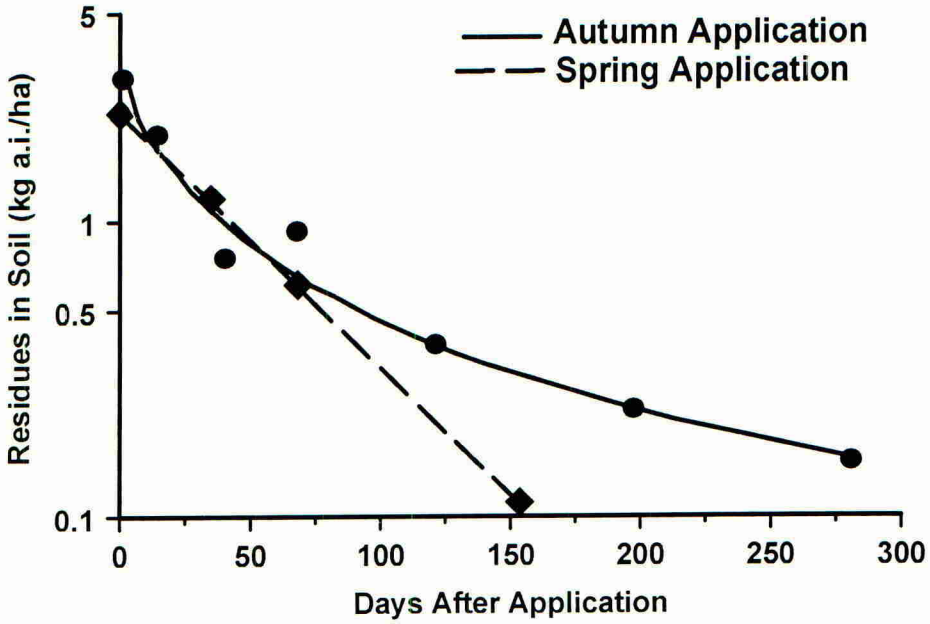
U.K. Cereal Herbicide Study

Monitoring studies performed by a variety of researchers and water companies have shown that agricultural chemicals applied in cereal growing regions of the U.K. are present in adjacent ditches and rivers (for example, Clark et al., 1991; Healey, 1991). The source of these inputs has been suggested to be a combination of spills and other point sources, spray drift, drains, and runoff. In order to investigate the relative importance of these mechanisms, a field study was established on a commercial field in Bedfordshire. A secondary objective of the study was to measure the degradation rates of a moderately mobile cereal herbicide (Koc of 125) following autumn and spring applications.

The study was conducted on a 28 ha field with a Hanslope cracking clay soil, a typical cereal growing soil in the U.K. Mechanical analyses showed the soil was composed of 40 percent sand, 15 percent silt and 45 percent clay. Organic matter was about 3.0 percent decreasing to about 0.5 percent 60-90 cm below the soil surface. Soil pH ranged from 7.6 in surface soils to about 8.0 in subsoils. Drainage tiles from the field site emptied into a ditch, which flowed throughout the year. Cereals were also grown on the field on the opposite side of the ditch, and the herbicide was applied to this field in the autumn on the same day as the test field. Upstream of the field was a military airfield, so the sole source of herbicides in the ditch water was the two adjacent fields.

In the test field, runoff samplers were installed in a portion of the field adjacent to the ditch where a continuous slope of up to 10-20 percent was present. Samplers were also placed on all seven drain outlets into the ditch. Each week during the nine month study period, runoff and drain samplers were checked and samples of ditch water were collected just upstream and downstream of the test field and about halfway between. In addition weekly samples were collected at an NRA sampling point on the River Kym, about 10 km downstream of the test site.

FIGURE 1. The decline in the amount of the cereal herbicide following autumn and spring applications.



At the start of November the entire field was planted with wheat and the herbicide sprayed onto the soil surface at the end of November at a rate of 2.5 kg a.i./ha. In early April, a second application of 2.1 kg a.i./ha was sprayed onto the third of the field adjacent to the downstream portion of the ditch. Plots 40 by 40 m were established in each of these two portions of the test site and sampled regularly to measure the degradation and mobility of the herbicide in soil.

Soil residues of the herbicide (Figure 1) in excess of the detection limit of 10 µg/kg were confined to the upper 30 cm of soil. Degradation rates calculated using the models of Gustafson and Holden (1990) and Timme *et al.* (1986) are shown in Table 2. Both models indicate shorter DT50 values following autumn applications. Conversely DT90 values were shorter following the spring application reflecting the period of warmer temperatures during the summer.

TABLE 2. Degradation kinetics observed following autumn and spring applications.

	Autumn	Spring
Gustafson and Holden model		
kinetics	non-linear first order	linear first order
DT50 (days)	22	35
DT90 (days)	166	117
Intercept (kg a.i./ha)	2.78	2.27
Timme *et al.* model		
kinetics	square root first order	linear first order
DT50 (days)	15	35
DT90 (days)	164	117
Intercept (kg a.i./ha)	2.98	2.27

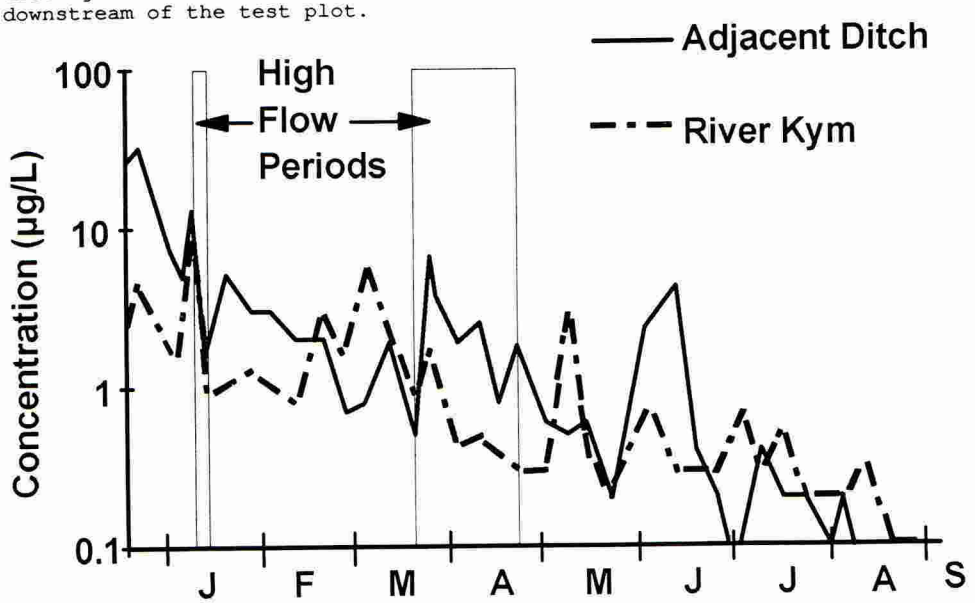
Concentrations of the cereal herbicide above the detection limit of 0.5 µg/L were observed in water samples from the adjacent ditch following the application until about the middle of August (Figure 2). The highest concentrations occurred prior to the first significant rainfall event and were probably the result of spray drift or the washoff of spray drift from the vegetation growing along the edge of the ditch. However, these higher concentrations cannot be equated with large inputs of the herbicide into surface water since the ditch was barely flowing prior to the significant rainfall in mid-January due to the drought conditions the region had been experiencing during the previous three years. The greatest amounts of the cereal herbicide entered the water during the periods of high flow indicated on Figure 2.

Residues of the cereal herbicide were found in water exiting the tile drains with concentrations highest in the first intense rainfall event following application, with little difference observed between autumn and spring applications. Although rainfall and tillage resulted in surface soils with no apparent cracks from the time of the autumn application until about a month after the spring application, the dryness of the subsoils indicated that flow through the soil profile was via macropores rather than the result of "classic" leaching.

During most of the study period, the amount of the herbicide entering the ditch via the drains was negligible, due either to lack of flow or the low concentration of residues in the drain water. During these times the residues in the ditch were probably the result of interflow. Soil cores collected during the study indicated that this interflow occurred mostly in the area of the field adjacent to the upstream portion of the ditch. The drainage network in this portion of the field appeared to be quite limited. These results suggest that perched water resulting from the macropore flow moves to the ditch even in the absence of drains, although the residues will be dispersed over a greater period of time than residues in water from drains.

During the study, no runoff of surface water was collected in the samplers, despite the occurrence of two intense rainfall events (a site visit during one of these events confirmed the absence of runoff). The

FIGURE 2. Concentrations of the cereal herbicide observed in the adjacent ditch just downstream of the test plot and in the River Kym about 10 km downstream of the test plot.



slope and heavy soil at this site were more conducive to runoff than many agricultural fields in this region, so the absence of runoff was a surprising result. Although runoff can potentially occur at any site, its absence during the study suggests that runoff is not normally as important as other transport mechanisms.

The concentrations in the downstream ditch were similar to the those measured at the downstream sampling point on the River Kym. Four periods occurred when residues in the ditch were significantly higher than in the River Kym. Two of these periods are probably related to site specific activities: spray drift following application, and the period in May and June that was influenced by the spring application (most applications of the cereal herbicide are made during the autumn in this region). The other two periods occurred following the significant rainfall event in mid-January and during the rainfall period at the end of March through the end of April. Residues at the test site would be expected to be higher during these conditions most favourable for transport of residues since residue concentrations in the water in the River Kym would represent an average over the entire region. The relatively good general agreement between the ditch and river concentrations indicate that the transport mechanisms resulting in residues in the ditch are probably also the most important mechanisms over the entire region. However, one mechanism not addressed in this study was point sources, such as spills and improper mixing and disposal practices. Research performed by Harris *et al.* (1991) indicates that point sources can be a significant contributor to surface water residues, especially for peak concentrations.

In summary, the study shows that the major source of herbicide residues in surface water in U.K. cereal growing areas is from water perched on subsurface soil layers being transported to adjacent ditches by drains or direct seepage. The highest inputs occur in the first intense rainfall event following application, with little differences observed between autumn and spring applications. These results are in agreement with other studies showing the appearance of a wide variety of agricultural chemicals in drainage water (Bird *et al.*, 1991; Bailey *et al.*; 1992; Catt, 1991; Matthiessen *et al.*, 1992; Harris *et al.*, 1991). Therefore, research into management practices to minimise transport of agricultural chemicals into surface waters in the U.K. should concentrate on methods for reducing rapid transport of water through the soil profile via macropore flow.

German Buffer Strip Study

Although runoff was not shown to be an important mechanism in the previous U.K. cereal study, runoff is an important mechanism for transport of agricultural chemicals under other conditions (Leonard, 1990; Wauchope and Decoursey, 1986). Buffer strips have been proposed as a management practice, but usually only for reducing losses of chemicals that strongly sorb to sediments. Experiments were performed with the same cereal herbicide used in the previously described study to determine the effectiveness of buffer strips with moderately sorbed chemicals that are present primarily in the runoff water (rather than the eroded sediments).

A series of five experiments was performed at the University of Karlsruhe using the rainfall simulator and procedures described by Michenfelder and Schramm (1992). Experiments were conducted on five plots, each 4 by 22 m with a slope of about 13 percent. The herbicide was applied to an area of 4 by 17 m in each plot. The 5 m wide untreated area was at the lower end of the plot in trials with buffer strips and at the upper end in trials without buffer strips. Except for the grass strip used in the spring experiments, all of the areas in all of the plots had been used to grow winter wheat, however, the seeding occurred just after the autumn trials. Each of the experiments consisted of measuring runoff losses during a single runoff event of 28 mm occurring the day after application of the herbicide to the plot. The duration of the runoff event was 40 minutes in the autumn trials and 60 minutes in the spring trials.

The autumn trials were performed on bare soil after tillage, representing a pre-emergence application of the herbicide. These trials

consisted of two plots, with and without a bare soil buffer strip. In the spring trials, the three trials consisted of a plot without a buffer strip, a plot with a five metre buffer strip of untreated winter wheat, and a plot with a five metre grass buffer strip. Results of the experiments are summarised in Table 3.

TABLE 3. Effect of buffer strips on runoff water and herbicide losses in the experiments performed at the University of Karlsruhe.

	autumn application		spring application		
	none	bare	none	crop	grass
Runoff Water (%)	24.6	24.2	21.8	21.9	3.2
Soil Erosion (kg/ha)	6100	5900	800	900	<5
Herbicide Loss (%)	0.8	0.5	2.2	1.2	0.05

Both the autumn and spring applications show that herbicide losses are less in the plots with buffer strips. Herbicide losses are reduced by about 40 percent with buffer strips of bare soil or wheat. The similar performance is not surprising since the soil surface beneath the wheat crop is essentially bare ground. These two buffer strips did not significantly affect the amount of runoff water leaving the plots or the amount of soil erosion. However, the grass buffer strip was much more effective, reducing herbicide losses by a factor of 40, reducing the water leaving the plot by about a factor of 6, and essentially eliminating soil erosion.

As expected, differences in runoff, erosion, and herbicide losses occurred between the autumn and spring plots with no buffer strip. The tillage that occurred prior to the autumn applications tended to reduce runoff and therefore, herbicide loss but greatly increase soil erosion. The increased rainfall intensity during the autumn trials would tend to increase runoff and therefore, herbicide loss, as well as to increase soil erosion.

Although more research needs to be performed under field scale conditions to optimise the size and performance of buffer strips, these experiments show that grassed buffer strips can be effective in reducing losses of agricultural chemicals in runoff water.

CONCLUSIONS

Field studies can be useful tools in assessing the behaviour of agricultural chemicals in soil and water. Uses of field studies include measurement of degradation rates under field conditions, determining the importance of various transport mechanisms, and development of management practices reducing residues in ground and surface water.

Field studies usually involve the collection and analysis of large numbers of samples. Therefore, regulatory guidelines for conducting field studies should be flexible so that efficient sampling programs can be designed to meet the objectives of the studies in accordance with chemical properties and site conditions.

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LYSIMETER STUDIES: DATA COLLECTION AND INTERPRETATION.

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ABSTRACT

The conduct of lysimeter studies at the Letcombe Laboratory will be breifly described including the selection and collection of soil types and characterisation in terms of their saturated hydraulic conductivities and the behaviour of passive tracers. The shortcomings of some of the management practices imposed by current guidelines will also be discussed. The conduct of studies, the use of radiotracers, the significance of trace polar components that concentrate in leaching water samples and the significance of the variability observed in replicated lysimeter experiments will also be discussed. Finally, the interpretation of lysimeter data and its use as a regulatory tool will also be considered.

INTRODUCTION

The regulatory requirement for lysimeter studies has now been with us since 1988 in the form of the BBA guideline IV, part 4-3 which requires candidate compounds to be tested in undisturbed soil monoliths maintained under normal agricultural practices and exposed to the "Hamburg average" rainfall total of 850mm. The required soil type is a sandy soil (>75% sand, <1.5% oc) which is the notional worst case for leaching. The protocol requires a minimum surface area of 0.5 m^2 and the lysimeters should be planted with appropriate crops for the compound being tested. At DowElanco we have now conducted lysimeter tests on 5 compounds, both at contract and within the Letcombe laboratory lysimeter complex and a number of these have now been reported (Brumhard *et al.*, 1992, Bergstrom, 1992). In this paper data from various experiments conducted at the Letcombe lysimeter are presented and discussed with reference to the characterisation of the hydrology of the lysimeters, the reproducibility of data from replicated treated lysimeters and the interpretation of these results with reference to the EEC drinking water Directive (Anon., 1980) threshold of 0.1µg.l^{-1} for individual pesticides within the regulatory framework.

EXPERIMENTAL

Soil selection and characterisation.

Three soil types have been sampled and collected for installation into the lysimeter complex at Letcombe. The soils are representative of the Cuckney series (sandy soil, in compliance with the BBA guideline), Oxpasture series (medium clay over loam) and Sutton series (uniform loam to a depth of ca. 80 cm). The soils were identified and

collected on behalf of DowElanco by the Soil Survey and Land Research Centre, Silsoe campus, Beds. The soil properties and their occurence in Europe have been reported previously (Yon, 1992). The latter 2 soils were selected as being representative of heavier, less freely drained agricultural soils of the type common in the UK and are of particular importance to aquifer contamination where they overly porous subsoils such as sandstone, limestones or fractured chalk, all of which may contain aquifers.

After a settling in period, the hydraulic characteristics of representative lysimeters of the 3 soil types were investigated by determining saturated hydraulic conductivity (Ksat) values. The water flow was further characterised using passive tracers (Cl^- and Br^-) after the method of Saffigna *et al.*, 1977. The lysimeters were saturated by watering from above and then the tracers were applied, one in the centre of the lysimeter and the second in a ring in contact with the wall of the glass fibre cylinder. The watering was continued and samples were collected and analysed using a Dionex ion chromatography system.

Study Design

Protocols were designed to investigate the behaviour of test chemicals in at least 2 soils (including the sandy soil) under a variety of typical agricultural scenarios reflecting different agricultural rotations and allowing multiple applications. This was done before such requirements were written into the guidelines in order to maximise the information gained from these very expensive studies. The use of radiotracers is recommended in the guideline so as to increase analytical sensitivity and maximise information obtained from these experiments.

The cultivation practices carried out on the lysimeters have been described elsewhere (Yon, 1992) and, to date, winter wheat, winter barley, field beans, sugarbeet and winter oilseed rape have been sucessfully grown. The crop is particularly important as it contributes to the upward movement of water in the form of evapotranspiration and this is particularly important in spring when plant growth is vigourous. Leachate is collected in response to rainfall and during the winter months is at least weekly and often daily. This was done in order to examine how the lysimeters leach in response to rainfall on a daily basis. Soil samples were collected to a depth of 50 cm after the end of the first growing season to minimise the disruption to the soil profile and to a depth of 1 m at the end of the experiment. Climatic data (rainfall, maximum/minimum air temperature and soil temperature at 10 and 30 cm depth) was also collected either manually or using a weather station with a data logger.

RESULTS AND DISCUSSION

Saturated hydraulic conductivity (Ksat) values were determined as 7.8-35.7 $cm.h^{-1}$, 1.8-6.3 $cm.h^{-1}$ and 22.3-23.7 $cm.h^{-1}$ respectively for the sand, mloc and loam and were of the correct order for these soil types (Hollis and Woods, 1989). This confirmed that the lysimeters were behaving as expected and their hydraulic characteristics had not been extensively modified during collection. Breakthrough curves for the Br^- and Cl^- tracers

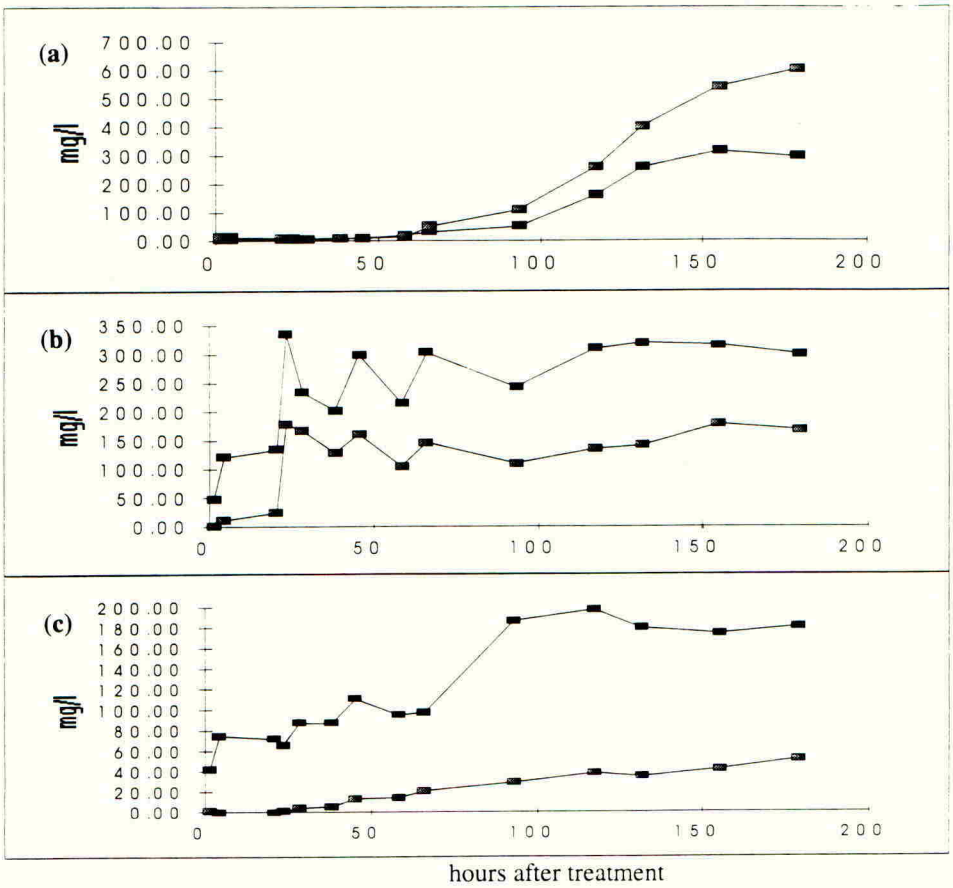

Figure 1. Breakthrough curves for (a) sand, (b) mloc and (c) loamy lysimeters. (Cl^- solid squares, Br^- hatched squares)

are shown in Figure 1. The fact that the Cl^- and Br^- reach the bottom of the lysimeters in the same water fraction indicates that there are no significant side wall effects. The shapes of the curves are also indicative of the mechanism of water movement through the lysimeters. Movement through the sand cores is by pore flow and is characterised by a lag time of 30-60 hours (equivalent to 9-20 litres of leachate) before the tracers appear in the water samples collected from the bottom of the lysimeters (see Figures 1a). Flow through the medium loam over clay, on other hand, is characterised by the tracers reaching the bottom of the lysimeters almost immediately (after 4 hours and 2 litres of leachate) and is consistent with by-pass flow as the mechanism of water movement once the soil profile becomes saturated (see Figure 1b). The tracer data for the loamy cores was confused by interfering peaks in the analysis of Cl^- ion but the Br^- was characterised by a lag time of 30-90 hours and the curve shape was characteristic of pore flow. The data serves to demonstrate the importance of understanding the water flow mechanisms at work in the

lysimeters as these may greatly impact the results. The experiment also showed a great deal of variability between different replicate lysimeters of each soil that were tested.

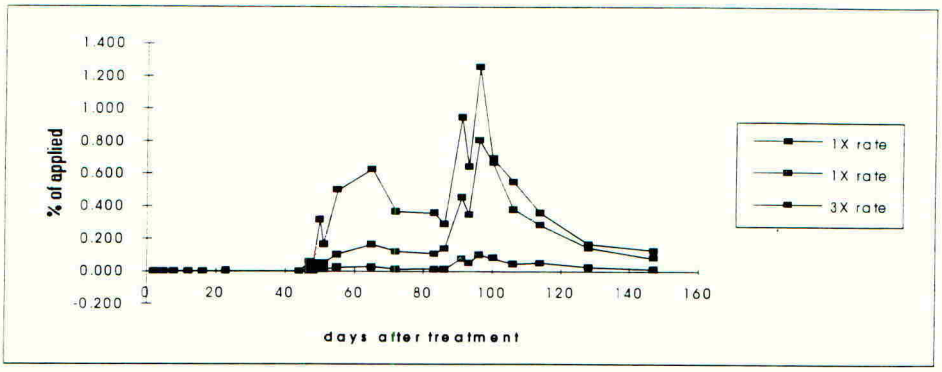

Figure 2. Radiotracer leaching response to rainfall.

Figure 2 shows the response of a 3 sandy soils lysimeters following the application of a radiotracer during the winter of 1989/90. The curves all demonstrate the onset of leaching after 40-50 days which is consistent with a pore flow mechanism but demonstate quite markedly different amounts of radioactivity leached during the first year indicating that there is a lack of homogeneity of the soil cores with respect to adsorption and degradation which impact greatly on leaching.

TABLE 1. Soil residue data after 2 years for an autumn applied compound.

Treatment rate	Residue ($\mu g.kg^{-1}$)		
	0-10cm	10-20cm	20-30cm
1X	9.6	10.3	3.7
1X	13.0	9.5	3.0
3X	19.0	24.4	20.7

The lack of homogeneity is further demonstrated in Table 1 which shows some data on soil residues of an autumn applied pesticide that remain in the 0-30 cm horizon of sandy soil lysimeters at the end of a 2 year experiment. The data shows a good degree of agreement between the duplicated treatments and also shows the expected dose related trends for the three treated lysimeters. Table 2 shows the annual average concentrations of the pesticide measured in the leaching water from the same 3 lysimeters for the first and

TABLE 2. Leaching water residue data for an autumn applied compound ($\mu g.l^{-1}$).

Treatment rate	Year1	Year 2
1X	0.69	0.003
1X	0.06	0.003
3X	0.87	nd

second years of the experiment. The data shows a wide range of concentrations ranging from 0.003-0.87 $\mu g.l^{-1}$. The dose relationship observed for the soil residues is absent. In total the percent leached represents only a very small proportion (<2%) of the applied dose.

Another interesting observation is presented in Table 3. The data represents the annual average concentrations of a metabolite in leaching water following the spring application of a pesticide to sandy soil lysimeters. The parent compound was absent from all leachate samples. As can be seen, the concentrations of the metabolite in the second year approaches $0.1 \mu g.l^{-1}$ and potentially, this is the moiety on which this product would be regulated.

TABLE 3. Leaching water residue data for a spring applied compound ($\mu g.l^{-1}$).

Treatment rate	Year1	Year 2
1X	0.03	0.09
1X	0.03	0.06
2X	0.03	0.07

This problem can be further compounded as demonstrated in Figure 3 which shows a chromatogram of a bulked leaching water extract from one of the experiments. The identity and concentration of components A-D known and none of the components exceed $0.1 \mu g.l^{-1}$. Components E and F, on the other hand are unknowns and their concentrations are approaching $0.1 \mu g.l^{-1}$ and, therefore, the decision was made to identify the

components. Several options were available for concentration of the samples including solvent extraction, freeze drying and solid phase cartridge concentration. The latter was selected for preparation of the samples and then the concentrated extracts were analysed by both thermospray and electrospray HPLC-MS. These components were not observed in the soil profiles from these lysimeters and in total represent <3% of the applied dose. The effort currently being put into their identification is totally disproportionate to the amount formed and serves to demonstrate how small amounts of very polar components can concentrate in the leaching waters. As the total mass of compounds involved is very small their isolation involves the extraction and purification of large amounts of water and, additionally, there is no guarantee of success. The question also has to be asked as to what to do in the absence of a structural identification and here, the researcher is lead down the road of having to demonstrate toxicity/no toxicity of the residues in the leaching water in the absence of any regulatory guidelines.

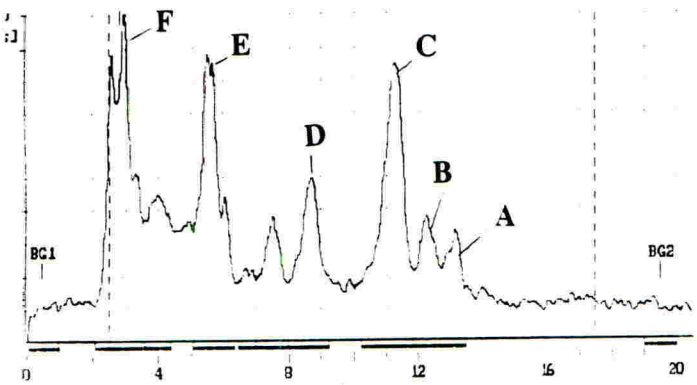

Figure 3. Chromatogram of a leaching water extract.

CONCLUSIONS

All of the data described above clearly demonstrates two things: firstly that soil is not a homogeneous medium when considered on the scale of replicate $0.5m^2$ lysimeters collected within a few metres of each other and secondly, because of this variability the results of lysimeter experiments can be very difficult to interprete in terms of fixed trigger levels. Conceptually, the variability of soils is not difficult to handle and at DowElanco, variability of climatic input, soil properties and chemical properties is a key feature in all of our mathematical risk assessment efforts (leaching, run-off and volatility). In our probability modelling (Laskowski et al., 1990) and in the EPA model PRZM2 (Pesticide Root Zone Model, Mullins et al., 1992), the variability of these properties is recognised and dealt with by describing soil and pesticide properties as distribution functions rather than individual values. Both models then conduct large numbers of simulations sampling input parameters from the distribution functions and the output data are statistically analysed and presented as the probability of an event occurring.

The regulatory issue is harder to deal with and has a number of facets. Firstly and most frequently discussed, the trigger value ($0.1\mu g.l^{-1}$) by which the results of lysimeter experiments are interpreted by certain Regulatory Authorities are not toxicologically founded, secondly it relates to drinking water and finally, only in rare occurrences of extremely shallow aquifers are lysimeters good models for groundwater and certainly in the UK our major groundwater sources are deeper than 1m (Cradock-Hartopp, 1977). At DowElanco we have undertaken a research project to attempt to bridge the gap in our understanding of lysimeter results and groundwater. At our research farm in Norfolk we have characterised the water movement within a small catchment (Hollis, 1991) consisting of a typical brown sand (Newport series) developed in sandy drift with underlying impermeable Gault clay at a depth of 3-6m. The depth to the winter water table is within 2m of the surface. Four $0.5m^2$ x 1m deep lysimeters were collected from the site and then buried back in situ in an experimental plot which represents only a small part of the catchment. Boreholes (to a depth of 4m) for the collection of groundwater samples were installed on the down slope side of the plot and water can also be collected from a small pond and stream which represent the outflow point of the catchment. A diagram of the experimental site is presented in Figure 4. Initially, the intent is to study the behaviour of passive tracers in the system and then to use a combination of existing leaching models and 3 dimensional finite element aquifer models to interpret the results, especially with respect to dilution of residues leaching from a field.

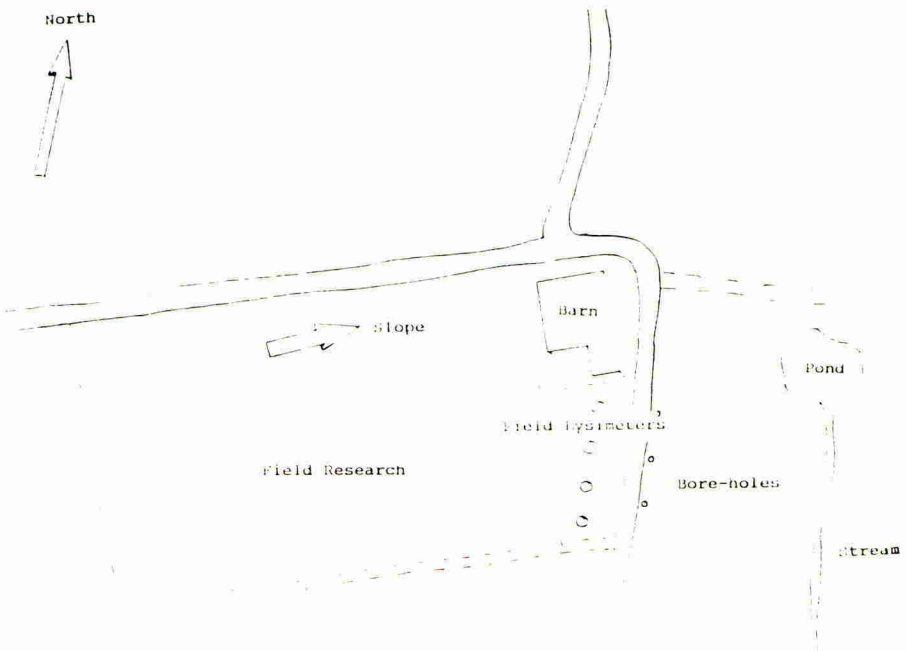

Figure 4. Diagram of the experimental leaching plot.

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HERBICIDE RUNOFF MEASUREMENTS FROM SMALL PLOTS: HOW REALISTIC?

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ABSTRACT

The amounts of preemergence-applied atrazine and alachlor lost in runoff water from 624 m^2 "mesoplot" and 5.57 m^2 "microplot" corn plots on a loamy sand soil were determined under identical, reasonable-worst-case conditions. A rainfall simulator was used to generate runoff from one mesoplot and two microplots simultaneously. The microplots and mesoplots appear to give similar herbicide losses. Total losses were rather small because of the large amount of infiltration in this soil: atrazine losses of 2%, and alachlor losses of 0.7% of applied amounts were obtained.

INTRODUCTION

Runoff--the loss of water and sediment from the surface of soils when rainfall exceeds infiltration--is an important route of transport of pesticides away from the site of application. This transport leads to observable traces of pesticides in ponds, streams and larger water resources, and ia a concern for exposure of aquatic ecosystems and humans (Wauchope, 1978; Leonard, 1990; World Wildlife Fund, 1991).

Procedures are needed to accurately assess the risks of pesticides in runoff, especially when new pesticides or uses are proposed. This requires, in addition to knowledge of the toxicity of the pesticide to potential nontarget biota, an estimate of the concentrations of the pesticides which runoff is likely to produce in water resources. Currently, field experiments, combined with research into processes and computer simulation modeling, allow estimates of runoff losses of pesticides with an accuracy on the order of a factor of two for a postulated situation--provided the situation is similar to one for which the models have been validated (Knisel, 1980; Donigian, *et al.*, 1986; Wauchope, 1992; Lorber and Mulkey, 1982). However, modeling extrapolations from one site to another have confidence limits so large that they are not very informative unless the results are extreme, i.e., indicate

either a wide safety margin or high risk. Thus, experimental measurements are often required.

Traditionally, runoff experiments have consisted of instrumenting a watershed, applying the chemical and waiting for rain. Unfortunately, runoff losses are very sensitive to the time elapsing between application and runoff: typically losses decrease by nearly a factor of 10 for each week of rain delay (Wauchope and Leonard, 1980. Thus, the traditional experiment may give little useful information (Hendley, 1992), since the information usually desired for risk assessment is for worst-case situations, and these will, of course, seldom occur.

This has led to the use of rainfall simulation experiments in which artificial runoff is generated within a day or less of pesticide application. Such experiments have generated much information, especially of a comparative nature. However, the absolute amounts of pesticides lost in these experiments have been viewed as artificially high. This is because the plots usually used have been small--typically a few m^2--and such small plot studies often give higher losses of pesticides than observed in field studies. However, a recent review of the literature has suggested that this difference may not be due to an inherent deficiency of small size plots, but due to the fact that such plots have typically been used with rainfall simulators, under worst-case conditions seldom seen in the field (Wauchope and Burgoa, 1993).

In this report we present initial results from an experiment in which a direct comparison is made between herbicide losses from 5.57 m^2 "microplots" and plots of 624 m^2, which we refer to as "mesoplots" because they are intermediate in size between microplots and typical fields.[1] Mesoplots are large enough to investigate tillage and crop effects and flow lengths can be used which produce rill erosion as in fields. We believe that mesoplots also show much promise in investigating mechanisms of losses of foliar-applied pesticides.

A comparison of mesoplots and microplots under identical conditions was possible because of the recent development of a convenient and realistic rainfall simulator capable of generating runoff on mesoplot-size areas (Coody *et al.*, 1992; Sumner *et al.*, 1993; Wauchope *et al.*, 1990). We used a simulator that covers a 20 m x 55 m area to rain simultaneously on a mesoplot and two microplots. Because we could control rainfall timing and intensity, we simulated "reasonable worst case" conditions: a 2-hour, 5-cm rainfall occurring 24 hours after herbicide application. Herbicide application method and

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[1]We use the term in analogy to the aquatic toxicologists' "mesocosm"--a constructed pond which is small enough to be controllable and replicatable but large enough to reproduce most of the processes of larger water bodies.

the topography of the site were also selected to maximize runoff. The soil selected, however, was a loamy sand which gives relatively little runoff.

METHODS

Two mesoplots A and B, each with two associated microplots C/D and E/F respectively, were established on adjacent areas in a field near Tifton, Georgia. The soil was a Tifton loamy sand with a 3% slope. This soil has a high hydraulic infiltration rate when freshly tilled but rapidly settles to form a denser, less permeable surface layer during rainfall. The soil was disked twice to 15 cm depth, deep turned with a moldboard plow to 30 cm depth, and beds were formed parallel to the slope by tractor wheel tracks spaced 1.8 m apart. One week before herbicide application a 5 cm rain was applied to obtain background samples and to give uniform soil moisture prior to application and planting. On the day before the rainfall/runoff event fertilizer and a nematicide were broadcast on the soil surface and the soil was rototilled to 10 cm depth, corn was planted in rows 0.9 m apart and alachlor (0.48 kg/l emulsifiable formulation) and atrazine (90% dispersible granule formulation) were broadcast at 2.8 kg/ha and 1.87 kg/ha active ingredient, respectively, in a tank mix in 187 l of water per ha.

The rainfall simulator (Sumner *et al.*, 1993) consists of 46 vertical aluminum-tube riser pipes of 4.0 cm i.d. and 3.05 m height spaced at 3.05 m intervals along two parallel 67 m-long irrigation lines spaced 14.6 m apart in the centers of two beds with seven beds in between. At the top of each riser is a pressure regulator and an irrigation sprinkler (Senninger Wobbler with a #13 nozzle) that has a droplet size distribution similar to natural rain. At 138 kPa and 20 l/min this sprinkler pattern will deliver a 2.5 cm/hr rainfall intensity with a coefficient of uniformity near 90%, depending on wind turbulence, on an area 20 m x 55 m.

We collected drainage only from the 15 m-wide area between the irrigation lines and used a soil berm at the upper end and a collector trough at the lower end of a 43 m-long area to form the mesoplots. A hydrologic flume and recorder were attached to the collector trough. Two microplots were located in the area above each mesoplot within the simulator area. The microplots were 3.05 m long and 1.8 m wide, formed by pressing metal dikes into the soil. In cross section each microplot consisted of two half-beds with a tractor wheel-track running down the center.

Twenty-four hours after the herbicides were applied a 2-hour, 5-cm rain was simulated. Runoff samples were collected by hand from the outflow from mesoplot and microplot collector troughs. In the microplots, runoff flow rates were determined from the time required to fill sample bottles. All samples were stored at 4C until analyzed.

Each water sample was weighed and the herbicides extracted without filtering using dichloromethane and separatory funnels. Extracts were evaporated in rotary evaporators and the residue taken up in methanol. Analysis was both by reversed-phase HPLC and capillary GC using a thermionic N/P detector. Recoveries of spiked runoff samples ranged from 85-120%.

RESULTS

The data reported here are a subset of a much larger experiment in which full-season (six events) runoff and leaching into the root zone of thirteen chemical species including pesticides and pesticide metabolites, nutrients and tracers, are being measured from two mesoplots and four microplots for two years in corn. We report here the results of only the first simulated rain after herbicide application in the first year (1992) of the study. These results should be considered preliminary. This was, however, the critical event for the year so far as runoff of the herbicides is concerned, and allows a comparison of two mesoplot runoff events with four microplot events.

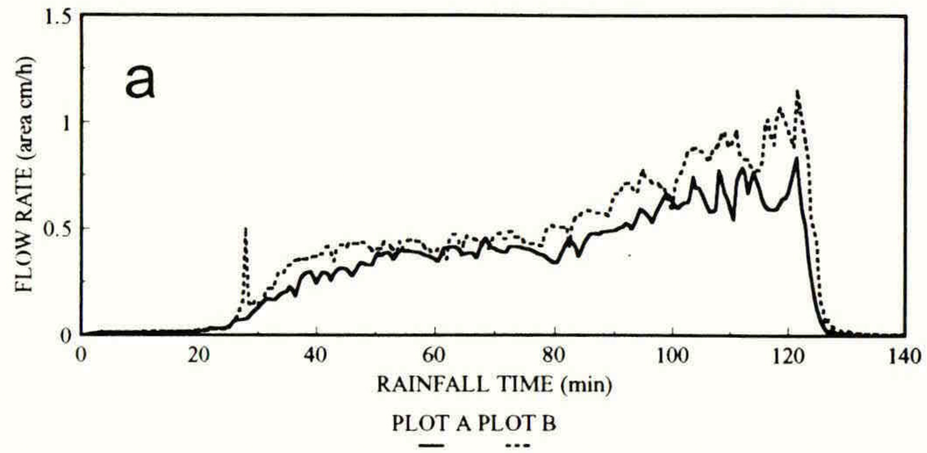
Hydrology

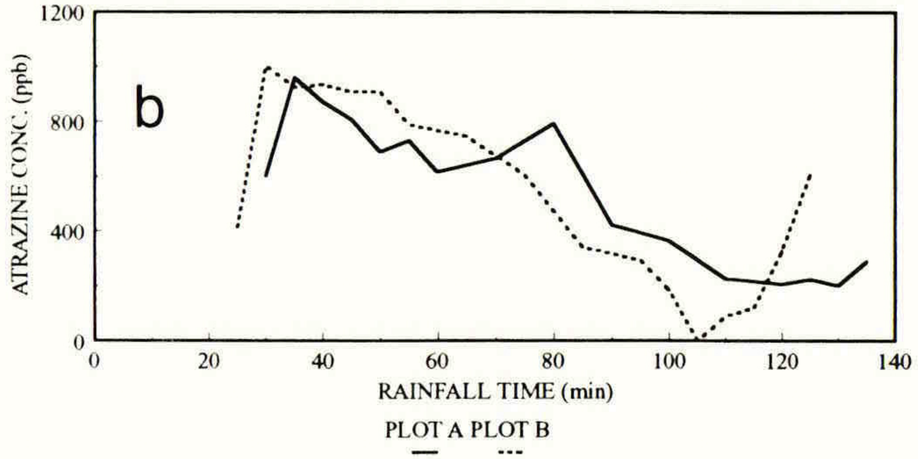
The mesoplot hydrographs (Figure 1a) show excellent reproducibility and, because of the constant intensity of the simulated rainfall, provide details in flow response not usually seen with natural rainfall. The small initial flow was established by rain falling directly into the runoff collector trough. After about 25 minutes of rain, runoff began from the tractor wheel tracks, which had been packed by 5 tractor passes and occupy about 20% of the plot area. This appears to establish a steady state of about 0.5 cm/h or 20% of the rainfall rate, by one hour. At about 75 min the tilled soil in the beds approached saturation and began to drain, causing a second period of flow increase. A second runoff steady-state was not established by the end of rainfall.

Rainfall events later in the season showed dramatic effects of increased runoff yields due to decreases in water storage space in the upper soil from settling during rains. Still-later rainfall events showed decreasing runoff as the crop canopy developed.

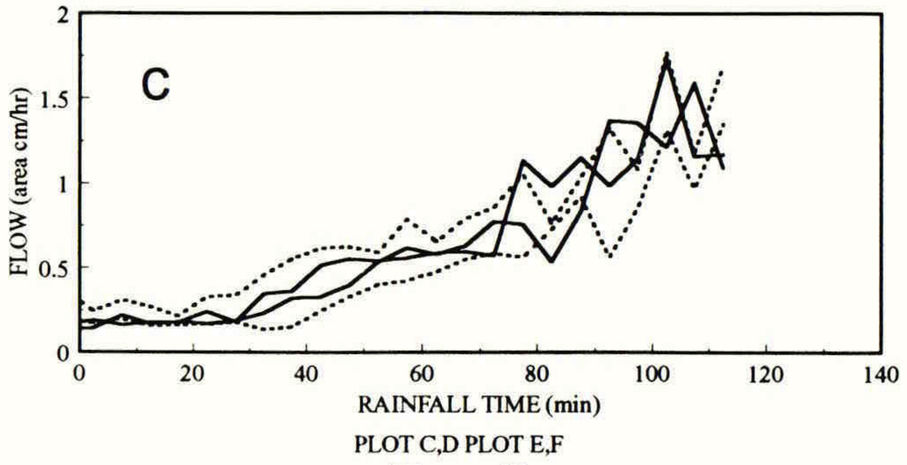
The microplots show similar hydrographs (Figure 1c), including the tractor wheel track/bed runoff sequence. Microplot F yielded significantly less water than the other microplots and may have had a ponded area. Maximum peak flows from the microplots are higher than the mesoplots on a unit area basis, but when the larger collector trough correction is made (approximately 0.2 area cm/hr), the difference ia about 25%.

MESOPLOTS





MICROPLOTS



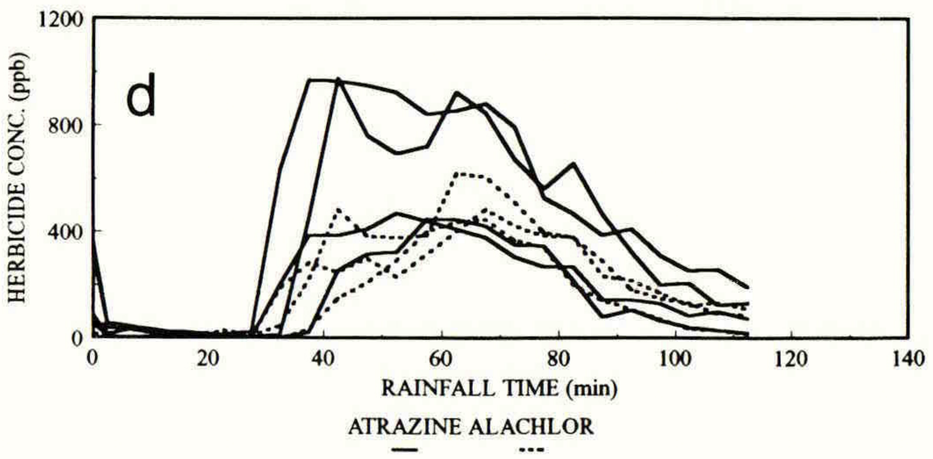


Figure 1. Water flows and herbicide concentrations, plotted against rainfall time. (a) mesoplot flows--note that rainfall rate is 2.5 cm/hr. Spike in plot B data is due to leak in irrigation pipe which was quickly repaired; (b) atrazine concentrations in mesoplot runoff ; (c) microplot flows--note large base flow due to rain in collector trough; (d) atrazine and alachlor concentrations in microplot runoff water.

Herbicide Losses

Atrazine concentrations in the mesoplots runoff (Figure 1b) are in good agreement and similar to those of microplots C and D (The higher pair of curves in Figure 1d), which were irrigated at the same time as mesoplot A. Microplots E and F, however, gave only about half the atrazine concentrations and yields of the other plots.

Alachlor analytical data are incomplete for this event and we will need to examine the 1993 data before reaching final conclusions. However, the three microplots for which we have alachlor data (C, E and F) are in close agreement.

Table 1. Total Water and Herbicide Losses from Mesoplots and Microplots in a 5 cm Runoff Event 24 h After Application

PLOTS	Mesoplot(624m^2)		Microplots (5.57m^2)			
	A	B	C	D	E	F
Tot. Runoff (l)	4382	5592	47.4	50.6	51.8	36.1
(cm)	0.702	0.896	0.85	0.91	0.93	0.65
Atraz. Loss (mg)	2090	2470	19.2	24.5	10.7	5.6
Alach. Loss (mg)	--	--	12.2	--	10.8	5.6
Avg. Atrazine Conc. (ppm)	0.48	0.44	0.41	0.48	0.21	0.16
Avg. Alachlor Conc. (ppm)	--	--	0.26	--	0.21	0.15
Atrazine Loss[1](%)	1.8	2.1	1.8	2.4	1.0	0.5
Alachlor Loss[2](%)	--	--	0.7	--	0.7	0.4

[1]Atrazine applied: 117 g/mesoplot, 1.04 g/microplot
[2]Alachlor applied: 175 g/mesoplot, 1.56 g/microplot

DISCUSSION

Our preliminary interpretation of these data is that both mesoplots and microplots exhibited similar hydrology and herbicide runoff losses--near 2% for atrazine and 0.7% for alachlor (we reported greater losses than this in an earlier, informal report of this experiment (Adams, 1993) due to a miscalculation of microplot runoff volumes). These herbicide losses are rather small considering the rainfall timing and intensity of this study. Wettable powders and emulsifiable concentrate formulations are generally expected to exhibit 5% and 1% losses, respectively, from fields under worst-case conditions (Wauchope, 1978). However, the soil used in this

study had an extremely high infiltration rate at the beginning of the storm. Thus, considerable leaching of the herbicides out of the soil/rainfall water interaction zone (Leonard, 1990) may have occurred before runoff began. Results from 0.5 m^2 tilted-bed experiments with the same soil, and herbicides (Burgoa and Wauchope, 1993) indicate that, in this soil under these conditions, much more herbicide movement occurs by leaching than by runoff.

If the 1993 data confirm the similarity in concentrations in runoff for both atrazine and alachlor between microplots and mesoplots, we will have strong evidence that even microplots may be useful to measure concentrations of pesticides in runoff, under some conditions. Such measurements could then be combined with hydrology/erosion models for much more accurate predictions of losses from larger areas than an a priori simulation of those areas. It is not certain, however, that microplot measurements can be used for foliar chemicals, and possibly not for sediment-borne chemicals. We are examining examples of these cases with other chemical species in this experiment.

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MEASUREMENTS OF TRACE RESIDUES OF HERBICIDES IN ENVIRONMENTAL SAMPLES - SOME NEW APPROACHES

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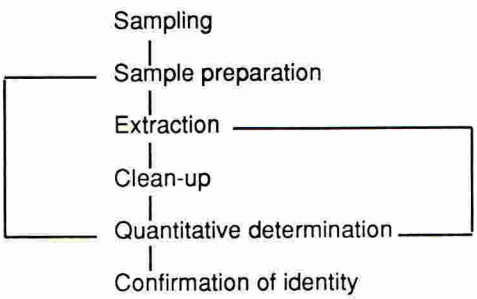
ABSTRACT

In support of legislative activity, analytical chemistry continues to play an increasingly important role in providing reliable data for surveillance of our food and the environment. New sampling and analysis approaches, geared to one ultimate goal, more efficient sample analyses, are being considered. Whereas most analytical schemes are currently based on gas and high performance liquid chromatography (GC and HPLC), various new technologies have entered the arena for serious consideration as routine methods. These are aimed at either achieving lower detection limits via improved sensitivity or selectivity of the detection system (eg atomic emission detection (GC-AED), immunoassay (IA), capillary electrophoresis (CE) and nuclear magnetic resonance (NMR) spectroscopy) or confirming the presence of residues with more confidence (e.g. using diode array detection (HPLC-DAD), mass spectrometry (HPLC-MS and GC-MS) and fourier transform infra red spectroscopy (GC-FTIR)).

INTRODUCTION

The need to evaluate the risk to the environment from the use of chemicals has been a significant part of the regulations to control pesticides for many years in the UK and elsewhere. There has been an increased awareness and concern from the public and regulatory authorities regarding the potential of pesticides to contaminate air, soil, food and water sources. Partly as a result of these demands and also due to an improvement in the discovery and development of new pesticides the limits of detection of pesticide residues in environmental samples are constantly being lowered. Older high dose rate products are re-evaluated or replaced by new lower dose rate ones, with a concomitant reduction in the environmental residue levels. All residue methods contain elements of some or all of the following stages in the following sequence:



Examination for improvements in each of these areas is ongoing, as evidenced by the extensive literature each year. Examples of the more interesting and promising developments are provided.

SAMPLING

In order to reduce the time and cost of sampling, a number of *in-situ* sampling devices and bioaccumulation approaches have been introduced to abstract and concentrate over time low levels of pesticides from water columns in the field

- membrane devices
- caged biological species
- adsorption columns/disks

Supported liquid membranes mounted in a flow system have been used to selectively extract and concentrate pesticides. Herbicides are extracted in their protonated form from acidified water samples with enrichment factors of several hundred fold. Such systems have been incorporated into field sampling devices to permit integrated sampling and quantitation at the lower ppt range (Mathiasson *et al*, 1991). Other approaches to time integration concentration of pesticides in aquatic systems have included the use of semi-permeable membrane devices (SPMDs) and the use of caged mussels or fish. The mussels/fish provide a measure of the bioavailability of the pesticide under test. Södergrun (1990) has shown that accumulation of lipophilic pollutants by the solvent filled dialyses membranes occurs in a similar way to that of the mussels/fish. The feasibility of the SPMD approach for *in-situ* monitoring has been demonstrated with an isomer of fenvalerate in an enclosure in a small pond (Huckins *et al*, 1990). The use of caged mussels or fish has been widely used in monitoring of pesticides and other organics in receiving waters. The uptake of pesticides and PCBs using the two approaches has been compared (Prest *et al*, 1992). Levels were slightly higher in mussels (~ 1.6 on a wet weight basis than in SPMDs, and trends in accumulation were similar. Gill *et al*, 1992 have compared the uptake of flufenoxuron from water using several devices with that of fish. The uptake using the passive *in-situ* devices were generally equivalentto that of the fish, though for ease of handling and analysis the C18 discs (EMPORE ^{TM}) were preferred.

A measure of the mean water concentration in a water body over time can be provided using a SEASTAR^{TM} sampling device. This periodically draws large volumes of water (for pesticide analyses) through extraction columns filled with adsorbent XAD resin. The efficiency of this device for extracting pesticides from water has been confirmed (Sarker and Sen Gupter, 1989).

EXTRACTION

Classically, water samples are extracted with organic solvents at appropriate pH values. However, their high solvent usage, slow speed and labour intensive nature makes them less suited to the modern analytical laboratory. Organic solvents must be disposed of safely and with minimum environmental impact. Ease of use and suitability for automation are increasingly important aspects of modern analysis and are probably the major reasons for the replacement of classical liquid-liquid extraction (LLE) by solid-phase extraction (SPE).

The extraction efficiency of LLE and SPE techniques are often comparable. SPE is generally applicable to a wide range of pesticides, though there have been few detailed studies which have statistically evaluated the recovery of selected pesticides on different SPE columns [eg recovery of diazinon on C8 was 83.5% + 2.3% and on C18 was 95.5% + 3.7% (Loconto, 1991)]. Practical problems of how to interface large sample volumes (typically a litre) with the cartridges to ensure reproducibility in an acceptable time have been addressed. Automated SPE is growing in popularity and a number of instruments capable of SPE are now available, which can automate column conditioning, sample addition, washing steps and subsequent elution of the sample. Automation of SPE has been achieved in Water Companies where large sample throughput is required for drinking water monitoring to meet the EC directive.

The majority of applications of enrichment of pesticides in water have involved the use of C8 or C18 bonded phase cartridges, particularly in multiresidue methods, e.g. triazole or pyrimidines (Bolygo and Atreya, 1991) chloroacetanilide and triazine herbicides (Meyer *et al*, 1993). USEPA have also included SPE for sample preconcentration in their test methods (EPA 500 and EPA 525). Treatment of water samples with acid prior to SPE on C8 cartridges is necessary to retain free acid herbicides (eg 2,4-D, dichloroprop and dicamba) (Bogus *et al*, 1990; Swineford and Belisle, 1989). Addition of methanol to the water sample can also significantly increase the recovery of more hydrophobic pesticides (Loconto, 1991). Less common adsorbents have successfully been employed for extracting pesticides from water samples. These include polyurethane foam, polymeric material PLRP-S (for phenoxy acid herbicides), Chromosorb 102, styrene-divinylbenzene absorbents, styrene-ethylenedimethacrylate copolymer Separon SF, Wofatit Y77, Aquapak 440A, florisil and amberlite XAD-2, 4, 7 and 8

Mixed mode isolation, which uses a combination of functional groups on a resin, can be used to simplify the co-isolation and purification of polar and non-polar, non-ionic analytes from water by taking advantage of dual mechanisms of isolation. A combination of octadecyl chains and sulfonic acid groups have been used for isolation and purification of triazine herbicides and their metabolites (Mills and Thurman, 1992). Another approach is to use two pre-columns in series. A tandem column approach (0.8g of C18 and 0.4g of phenyl bonded silica has been used for the multiresidue analysis of 50 pesticides (Benfenati *et al*, 1990). Two columns, C18 (to remove interferences) and the copolymer PRP-1 (which has a high affinity for polar phenylureas) enabled isolation and detection of phenylureas down to 0.01µg/l in a 500 ml sample (Hennion *et al*, 1990).

A non-specific adsorbent (Carbopak B) made from graphitized carbon black has proved to be more efficient than C18. It can adsorb both basic and acidic pesticides from water at any pH (Di Corcia *et al*, 1989). Fractionation of pesticides can then be achieved by eluting the cartridge with a range of solvents of different polarity or pH. This multiresidue approach has enabled extraction and quantitation of 89 pesticides in municipal and ground water samples below 0.1 µg/l (with a few exceptions).

Solid phase extractions can also be performed using adsorbent disks, eg EMPORE™, which comprise of chemically bonded silica particles individually suspended in a densely woven 'web' of micro PTFE fibrils. The disks are used with standard filtration equipment and a range of matrices, incorporating C8, C18 and a styrene-divinylbenzene polymeric matrix (for phenolic compounds) are available. One approach to improving the reliability of environmental water sampling procedures, particularly where there may be uncertainty of the stability of pesticides of interest is to extract water samples onto SPE material in the field and store the SPE material. Pesticides have equivalent or greater stability on SPE disks compared to storage in water at 4°C (Senseman *et al*, 1993) with freezing the SPE material after pesticide extraction being the most favourite option.

Although SPE as a sample extraction process is efficient in improving sensitivity, the selectivity, particularly with the non-polar stationary phases is not optimum. An alternative is to use pre-columns packed with phases containing immobilized antibodies (eg immunoaffinity chromatography). Immunoaffinity chromatography exploits the specific interaction between an antibody and its antigen to purify and concentrate the antigen from a dilute solution. These systems are usually designed to isolate one specific analyte, though an antibody reagant selected to exhibit a broad, or class specificity, may permit the isolation of a class of pesticides or metabolic products, which in such cases may be more useful. Class specific immunopurification has been successful in selectively absorbing chemicals containing a 2,4-dinitrophenyl group from an aqueous cocktail containing related chemicals (Aston *et al*, 1992). A recent and novel application of SPE is solid phase microextraction (SPME). A solid phase in the form of a fibre is held within a syringe which is exposed to the sample when the plunger is depressed. Pesticides and other organics can be adsorbed from solution onto the fibre and then analysed by thermal desorption. SPME is a fast, sensitive, inexpensive, portable and solvent-free method for extractingorganic compounds from aqueous solution. It can attain detection limits of 15 ppt and below for volatile and non-volatile compounds. (Arthur *et al*, 1992).

QUANTITATION

Selective gas chromatographic detectors are an essential part of the methods used to analyse pesticides in environmental samples. However, several different conventional detectors are required for selective detection based on all the heteroatoms (except oxygen) common to most pesticides. Atomic emission detection is a sensitive multi-element detection method for gas chromatography which offers several advantages over the more conventional detectors (Lee and Wylie, 1991). Any element except helium can be detected and its atomic identity confirmed with recorded spectra. Analysis time is fast since four or more elements can be recorded simultaneously. Since the response is essentially compound independent for

most elements, the concentration of an unknown can be quantitated, which permits the use of the detector as a screening tool as well as for trace analysis. Using standard solvent extraction and concentration techniques levels of 0.1 µg/l can be achieved for a number of pesticides using several different elements for selective detection.

The application of capillary electrophoresis (CE), particularly isotachophoresis and zone electrophoresis, to the separation and determination of organic molecules in a wide range of matrices is rapidly expanding, although there is as yet limited success in the area of pesticide chemistry (Kuhr and Monnig, 1992). Triazine herbicides (eg prometryne, tebutryne, desmetryne, simazine and atrazine) and their solvolytic products have been separated using capillary zone electrophoresis and detected by UV, though a limiting factor was the sensitivity (lod of 2x10-6M; Foret *et al* 1990). Other pesticides (eg prometon, prometryne, propazine and butachlor) have been determined at lower detection limits (18-52 fmol) using micellar electrokinetic capillary chromatography (Cai and El Rassi, 1992). More recently, two sulphonylureas, metsulfuron and chlorsulfuron were analysed by CE in water (Dinelli *et al* 1993). Using SPE and concentration (10,000 fold from 1 litre to 100 µl), 0.1 ppb of each compound could be detected using UV at 214 nm, though the electrophoregrams indicated the presence of some interfering compounds that arose from the SPE procedure. Isotachophoresis has been used for the detection of the pyrethroid insecticides, alphamethrin and cypermethrin in water. The insecticides are extracted from water, evaporated and then hydrolysed at alkaline pH to yield the degradation products, *cis* and *trans* dichlorochrysanthemic acids, which are separated and detected by capillary isotachophoresis to the 0.1 ppm level (Dombek and Stransky, 1992). The drive to obtain additional chemical information about molecules separated by CE continues to encourage the coupling of CE with mass spectrometers. On-line CE-MS under tandem MS conditions has given full scan spectra from 30 pmol levels of a range of sulphonylureas (Garcia and Henion, 1992).

Thin layer chromatography has found favour for screening for pesticide residues in water because of its simplicity and minimal cost. Separation and visualization systems have been identified for several pesticide groups including triazines, organophosphates and pyrethroids with detection limits in the range of 0.1-1 µg per spot. Other workers have developed TLC systems for a wide range of pesticides. Ambrus *et al* (1981) using single solvents and visualisation modes including o-tolidine plus KI, p-nitrobenzene-diazonium-fluoroborate, silver nitrate and UV radiation, p-dimethylaminobenzaldehyde and bioassays with fungi and enzymes were able to detect 188 pesticides. Similar visualisation reagents were used by Gardyan and Thier (1991) though with more modern techniques for spotting, separation (high performance TLC, HPTLC) and evaluation, including computer assisted densitometry, for direct quantitation of 150 pesticides.

An HPTLC method using automated multiple development (AMD) for multi-component analysis of pesticides in water has been developed by Burger *et al* (1990). The conventional isocratic elution (of TLC) has been replaced by multiple and step wise development combined with gradient elution. Screening and confirmation gradients, coupled with reflectance spectroscopy (multiwavelength scanning) and post chromatographic derivatisation, make it possible to detect pesticides in ground water and drinking water supplies to a limit of detection of 20 ng/l. At least 100 pesticides can be checked for their presence on one TLC plate. Improvements in the sensitivity, linearity and speed of the method have been gained by reducing the thickness of the silica gel layer of the TLC plates and by decreasing the gradient step increments.

Nuclear Magnetic Resonance Spectroscopy (NMR) is now considered sufficiently sensitive for the analysis of phosphorus and fluorine containing pesticides in soil and food products. Organophosphorus pesticides are widely used in agriculture and approximately 10% contain fluorine, though it is now increasingly common to find this atom in more recently synthesized structures. It is feasible to use NMR for screening organophosphorusinsecticides in crop and soils at residue levels ~ 0.1 mg/kg without clean-up of the extracts with only 30 minutes of acquisition time on a 400 MHz instrument (Mortimer and Dawson, 1991a). As the receptivity

of the fluorine nucleus is 12.5 times greater than that of phosphorus, it is possible to measure residue levels of fluorinated pesticides at 0.01 mg/kg under similar conditions. Any fluorinated pesticides which contain multiple equivalent fluorine atoms (ie CF_3 atoms) should be detected with more sensitivity. Trifluoralin has been detected at 1 mg/l directly in liquid samples (ie wine) (Mortimer and Dawson 1991b); a detection limit of ≤1 ppb in water following typical solvent extraction/concentration steps from water is therefore feasible. The advantages of NMR for residue analysis include the acceptable scan acquisition times (~ 30 min cf. typical GC or HPLC chromatographic runs), its selectivity and non-destructive analysis as well as the time saved as no clean-up is required. The main disadvantages are that it is compound specific and the detection limit can still be a problem.

Chemical sensors for environmental monitoring of pesticides are under active development, though at present they have limited value. Sensors which work by current or potential formation include ion selective electrodes and their combination with an enzyme substrate reaction has some application for the detection of organophosphorus compounds and carbamates. These substances, which are cholinesterase inhibitors, can be detected by correlating the cholinesterase activity with intermembranal pH shifts induced by substrate hydrolysis. Stein and Schwedt (1992) have applied an acetylcholinesterase biosensor consisting of a pH electrode and an enzyme membrane for screening of a number of pesticides in drinking water to the limit of 0.1 µg/l. Applications of chemical mass balances such as the piezo-electric (PZ) transducers have been used for solution control, eg with immobilized antibodies for atrazine and parathion detection (Guilbault and Schmid, 1991). Detection limits in the lower ppb range have been claimed.

Chemical fibre-optical sensor systems (FOCS) would appear to have a bright future as they are profiting from the rapid developments in the communications industry. The detection of fluorescent compounds (eg PAHs) is particularly widespread with the advantage of continuing time resolved fluorescent emission spectra with chemometrical methods for on-line and *in-situ* analysis of multicomponent mixtures. The permanent installation of a bare fibre configuration or a chemically reactive sensor tip should be an attractive solution for monitoring drinking water wells. Adelhelm *et al* (1992) used laser induced photo-acoustic spectroscopy with a UV exitation laser beam (253 and 376 nm) transmitted to a sample cuvette through a 600 µm diameter optical fibre to detect pesticides and polycyclic aromatics. Bioanalytical approaches of FOCS include the combination of cholinesterase inhibition and FOCS detection for organophosphorus compounds and carbamates (Hoebel and Polster, 1992).
These remote sensors have application in the area of continuous monitoring of ground water in wells and drilling holes. The method could give a real time indication of contamination and would therefore provide considerable time-saving over methods involving conventional sampling and subsequent laboratory analysis.

There has been a dramatic expansion in the application of immunoassay technology to environmental monitoring in recent years. It was hoped to eliminate the tedious, time consuming preparative work of conventional analytical methods by exploiting the specific molecule recognition and binding properties of antibodies for their analytes. In addition, the simplicity and versatility of immunossays lends itself to field test kits, which would be of particular value in environmental monitoring studies.

A considerable amount of development work has been undertaken by many groups to apply immunoassay techniques, in particular enzyme linked immunosorbent assays (ELISA) to the determination of pesticide residues in environmental samples, especially soil and water (Aston *et al*, 1992) The ELISA format is typically based on a 96 well micro-titre plate, which has the advantage of allowing some 20-30 duplicate samples to be screened simultaneously in addition to standards and QC checks. Owing to the selectivity of the antibody response, sample preparation can often be minimal. The sensitivities of many assays are such that a limit of determination for residues in water in the range 0.01 - 0.1 µg/l can be determined following solid phase extraction from a volume of about 150-250 ml. These features allow ELISA to be used for screening larger numbers of samples simultaneously than with

instrumental techniques such as GC or HPLC. The simplicity of the technique allows the construction of field test kits for some compounds. Current disadvantages of ELISA are the considerable investment of time and facilities needed for assay development and the slow acceptance of immunoassay procedures for generating pesticide data for regulatory use. There is only limited regulatory experience in the consideration/use of immunoassay data (eg US EPA) and there are as yet no formulated rules.

The number of immunoassays that have been applied to the detection of pesticide residues in water samples, although restricted in comparison to traditional chromatographic methods is steadily increasing. At present over 30 individual pesticides can be analysed. Comparative data shows the good comparability of ELISA and instrumental methods are being obtained, though accuracy and precision may be inferior to the instrumental methods. Common sources of error in drinking water analysis are due to cross reactivities and matrix effects; ELISA is prone to false positives. Validation of ELISA for routine analysis by independent methods and interlaboratory ELISA assessment is considered important.

There are reservations concerning the quality of some kits on the market, which fail to perform consistently with the manufacturer's claims. The area of greater concern is the lack of information on antiserum specificity. Moreover problems of sample matrix effects, which invariably have a significant influence on assay performance, are not always addressed by some manufactureres. Antibody quality is a key factor and with advances in antibody production and selection procedures, improvements in the performance of commercially available kits is expected.

CONFIRMATION

A number of techniques for providing spectroscopic data to aid confirmation of residues have recently gained prominence. These include diode array detection (HPLC-DAD), mass spectrometry (HPLC-MS and GC-MS) and Fourier transform infra-red spectroscopy (GC-FTIR).

GC-FTIR

Gas chromatography combined on-line with spectroscopic detection systems is a powerful technique for the rapid detection and characterization of organic compounds. Mass spectrometric detection systems are capable of distinguishing between homologues and of giving molecular weight and chemical structure information from fragmentation patterns. By contrast the infra-red spectrometer is capable of providing information on the presence of functional groups, positional substitution and can differentiate between isomers, but is less likely to distinguish between homologues. The spectroscopic data available from mass and infra-red detection systems is therefore complimentary and the acquisition of both types of data adds both scope and confidence to the characterization of unknown components.

Recent advances in Fourier transform infra-red (FTIR) methodology allow the identification of trace components on-the-fly at the low nanogram level. Despite this lack of sensitivity, many applications of GC-FTIR in environmental analysis have appeared. The US EPA has developed protocols (Method 8410) for the GC-FTIR analysis of semiviolatile organics including some pesticides (eg hexachlorobenzene, pentachlorophenol) (US EPA, 1989). Malissa *et al*, (1990) have demonstrated that the minimum identifiable concentration of atrazine in drinking water using the light-pipe technique is approximately 1 ppb; levels at the EC limit of 0.1 µg/l can only be achieved using cryodeposition techniques. Detection down at the low picogram level is possible with matrix isolation. The sensitivity and selectivity of GC-FTIR has enabled the technique to be successfully applied to the analysis of dioxin residues where the need to distinguish between individual isomers is paramount (Gurka *et al*, 1986).

HPLC-DAD

The application of HPLC to pesticide residue analysis is growing, particularly for those compounds which cannot be analysed by GC due to their polarity, poor volatility and/or thermal instability (eg phenylureas, carbamates). Increased attention on the positive presence

of polar pesticide metabolites in surface and ground waters has also been responsible for the growth in HPLC methods.

One of the fundamental weaknesses of liquid chromatographic techniques is that the standard detection devices (UV, fluorescence, electrochemical) have provided limited qualitative or confirmatory information. Retention time is insufficient nowadays to confirm the presence of an unknown and the problem is particularly severe for trace analysis of complex mixtures. Diode array detection (DAD) as an extension of conventional UV absorption detection offers good selectivity and sensitivity and has consequently found wide application in HPLC method development and analysis for peak identification and tracking. The structured and distinct UV spectral information from DAD has permitted workers to differentiate between chlorotriazines and their metabolites (Durand and Barcelo, 1989), paraquat/diquat (Simon and Taylor, 1988), and phenylureas (Reupert and Ploger, 1989) and it has been used as a standard method for monitoring surface, ground and raw waters in Germany (Friege and van Berk, 1989).

LC-MS

Although HPLC has been used for the determination of pesticides using UV, electrochemical and fluorescence detection the use of MS as an HPLC detector is considered a key element for the future of most HPLC methods. The confirmation of analyte identification provided by full scan EI-MS detection is invaluable to any laboratory.

The commonly used LC/MS interfaces include thermospray (TSP), particle beam (PB) and atmospheric pressure ionization (API). Thermospray HPLC-MS has been used to screen for relatively non-volatile pollutants in water including a number of polar herbicides and insecticides that are difficult to analyse by GC. Detection limits were in the range 1-10 µg/l for 29 analytes. Hammond *et al*, (1989) also used HPLC-MS for analysis of uron pesticides in river water. It is a relatively soft ionization technique and adducts for selected pesticides include $[M+H]^+$, $[M+H-CH_3]^+$ and $[M+H-Cl]^+$. By concentrating extracts (1 litre to 100 µl) and injecting 20 µl onto the column, Hammond *et al* (1989) were able to detect pesticides at concentrations equivalent to 0.1 µg/l in water samples in the selected ion monitoring mode (SIM). Barcelo *et al* (1991) have also identified characteristic base peaks and fragment ions for representative contaminants, chlorotriazines, phenylureas, phenoxyacids and organophosphorus quaternary ammonium compounds under positive and negative ion mode for thermospray HPLC-MS. Generally normal phase eluents give more structural information and enhance the response of several compounds when compared to reverse phase HPLC. Tandem mass spectrometry (TSP - LC/MS/MS) has also been used as a rapid screening method to detect hydroxy-simazine and hydroxy-atrazine at a limit of detection of 0.03 µg/l as part of a triazine groundwater monitoring study (Cornacchia *et al*, 1993)

The general lack of sufficient structural information from TSP spectra has encouraged the use of EI spectra generated from particle beam HPLC-MS for confirmation of pesticide residues. Generally PB-LC/MS is less sensitive than the other approaches mentioned though Miles *et al*, (1992) have shown that forty-three out of a hundred relatively polar National Pesticide Survey analytes have sufficient sensitivity using PB-LC/MS for detection of and spectrum generation from 100 µg or less, which is sufficient for confirming the presence of these analytes in groundwater at the 0.1 µg/l level. A PB-LC/MS method for chlorinated phenoxy acid herbicides in water has been developed as an alternative to the original GC method with diazomethane derivatisation (Brown *et al*, 1991). After extraction, the esters are hydrolysed to the free carboxylic acids, separated by reverse-phase HPLC and quantitated by UV with confirmation by PB-MS detection. No thermal degradation of these compounds in a PB mass spectrometer has been observed and full scan EI spectra obtained from 20 ng total material.

There are a number of atomospheric pressure ionization (API) interfaces, such as electrospray and heated nebulizer, in which the actual formation of ions occurs outside the vacuum system of the instrument. Electrospray (a soft ionization technique has been interfaced with an ion trap mass spectrometer (HPLC/MS/MS) (Lin and Voyskner, 1993). The

ability to acquire full scan spectra on picogram quantities of material (eg propoxur, carbofuran and aldoxycarb) enables the detection of 1-10 ppb levels of these pesticides by direct injection of 10 µl of the water sample into a column equilibrated in 100% water.

Electrospray LC/MS at high flow rates (2 ml/min) has been achieved by adding a simple liquid shield between the sprayer and the ion-sampling capillary of a heated capillary-type API interface (Hopfgartner *et al*, 1993). This has resulted in improved sensitivity and gives the benefits (eg ruggedness, efficiency, large injection volumes) of using standard HPLC columns. Applications have included the analysis of carbamate pesticides at the low nanogram levels (with conventional HPLC gradients) amd mexacarbate in pond water at 100 ppt level using selected ion monitoring (SIM).

API interfaces gave lower detection limits for analysis of carbamate pesticides in water in comparison with TSP and PB techniques (Pleasance *et al*, 1992). Doerge and Bajic (1992) evaluated atmospheric pressure chemical ionization (APCI) on analysis of four classes of pesticides (triazines, phenylureas, carbamates and organophosphorus compounds) plus some miscellaneous compounds in ground water. Detection limits in full scan or selected ion monitoring mode varied from 0.8-10 ng or 0.01-1 ng respectively with linear calibration curves. APCI-LC/MS is well suited to multiresidue pesticide analysis; it provides the optimal combination of high sensitivity,exclusive formation of protonated molecular ions and broad specificity across chemical classes.

CONCLUSION

The choice of analytical techniques for trace analysis of pesticides has broadened enormously over the last few years and has assisted in improving the efficiency (and hence cost) of sample analysis. These improvements have included promising developments in sampling and sample extraction/clean-up, as well as wider use of more selective detection systems and novel interfacing approaches (particularly in LC/MS). Further improvements can be achieved by extending standard multiresidue methods to include these novel techniques and applying them to a wider range of pesticides.

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