

# SESSION 4B

## MODE OF ACTION AND METABOLISM OF HERBICIDES: II

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## HERBICIDE DETOXIFICATION AND SELECTIVITY

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## ABSTRACT

Herbicide selectivity is determined by several distinct factors, such as rates of absorption into the plant and subsequent translocation, subcellular localisation, variation in target site sensitivity and the metabolic detoxification to less toxic derivatives. Though in many cases of selective action a number of these factors probably contribute to the different species responses, there is evidence to suggest that differential metabolism may constitute the most important factor for such herbicides as triazines, triazinones, aryl propionic acid esters, sulphonylureas, imidazolinone aryl carboxylates, propionanilides, methylphenylureas and substituted diphenylethers. Much of this evidence implicating the importance of differential metabolism between species with respect to selectivity is reviewed. Notable varietal reactions which may have a basis in herbicide metabolism are also discussed. Reference is also made to the usefulness of plant cell cultures in studies of pesticide metabolism by plants.

## INTRODUCTION

Herbicides recommended for the selective control of weeds have been developed to exploit a difference in phytotoxicity between species adequate to kill competing weeds without significantly reducing crop yields. In some cases the margin of selectivity may be quite modest and can be rendered inadequate when the timing of application coincides with unfavourable climatic conditions, as was reported to be the case for some of the phenylureas in winter wheat in Autumn 1983. There are a number of factors which can contribute to herbicide selectivity including soil placement, rates of absorption and subsequent translocation, localisation (both within the plant and at the sub-cellular level), transformation to products of modified phytotoxicity and differences in target site sensitivity. The selective properties of herbicides often result from a complex interaction of a number of these factors though there are many examples where one dominant factor has been implicated. This is certainly true in the case of herbicide uptake and movement (Hess, 1985) and differential metabolism to less phytotoxic products. The mechanisms by which plants transform foreign compounds have been adequately reviewed previously (Lamoureux & Frear, 1979, Shimabukuro, 1985, and Cole *et al.*, 1987) and include oxidations resulting in aromatic ring and alkyl hydroxylation, N-dealkylation, O-dealkylation and sulphoxidation, hydrolytic reactions, deamination and conjugation with carbohydrate residues (glycosidation) or the tripeptide glutathione.

## MECHANISMS OF DETOXIFICATION

Aryl hydroxylation

Ring hydroxylation represents a very important detoxification reaction in higher plants (see e.g. Cole 1983). However, species differ in their ability to aryl hydroxylate particular parent structures. Such is the case for bentazon for which a 200-fold selectivity margin between rice and Cyperus serotinus is attributable to rapid conversion in rice to 6-hydroxy

bentazon followed by glucose conjugation (Mine, Miyakado and Matsunaka, 1975). Metabolism in *C. serotinus* on the other hand is slow as is the case in other susceptible weed species (Mine *et al.*, 1975; Penner 1975). Rapid production of water soluble glycosidic metabolites also occurs in tolerant maize, navy-bean (*Phaseolus vulgaris*), barnyard grass (*Echinochloa crus-galli*) (Mahoney & Penner, 1975; Mine *et al.*, 1975) and wheat (Retzlaff & Hamm, 1976) whereas in soybean both the 6- and 8-hydroxy derivatives are formed (Otto *et al.*, 1979). Differences in tolerance to bentazon between soybean cultivars have also been attributed to relative rates of ring hydroxylation (Hayes & Wax, 1975) though metabolites were not characterised in detail.

Ring hydroxylation also plays an important role in the selectivity of diclofop-methyl between resistant cereals and susceptible grass weeds. Subsequent to rapid hydrolysis of the ester the resulting phytotoxic diclofop acid is inactivated by hydroxylation of the dichlorophenyl ring followed by glycosidation (Gorbach, Kuenzler & Asshauer, 1977; Jacobson, Shimabukuro & McMichael, 1985). Hydroxylation occurred at all three available positions on the ring though the 5'-hydroxy derivative predominated. In contrast aryl hydroxylation is of minor importance in susceptible oat and wild oat (*Avena fatua*) in which diclofop is primarily converted to the neutral glycosyl ester. Though glucose esters of acidic herbicides might be expected to accumulate in cell vacuoles, as for glycosidic conjugates, the susceptibility of oat and wild oat to diclofop-methyl implies that in this case compartmentalisation of metabolites is inadequate to confer tolerance. Susceptibility presumably results from the lability of the glucose ester. Interestingly, though diclofop-methyl has the same ring chlorine substitution pattern as 2,4-D there is no indication that aryl hydroxylation of diclofop-methyl occurs via the 'NIH' shift mechanism seen in 2,4-D. Indeed an 'NIH' shift has not been recorded for ring hydroxylation of any other pesticide in plants, including the related phenoxyalkanoic acid herbicide MCPA which is hydroxylated at the ring-methyl substituent (Cole & Loughman, 1983).

Despite the remarkably high biological activity of the more recently introduced sulphonylurea herbicides the margin of selectivity between cereals and sensitive weeds can be very large, approaching some 4000-fold in the case of chlorsulfuron (Sweetser, Schow & Hutchinson, 1982). Neither herbicide uptake nor translocation by tolerant (wheat, barley, wild oat and *Poa annua* annual meadow grass) and susceptible (cotton, soybean, mustard and sugar beet) species correlated with selectivity which was attributed to a striking difference in ability of young plants to oxidise chlorsulfuron to the 5'-hydroxy derivative which accumulated in tolerant species as the *O*-glucoside (Sweetser *et al.*, 1982). The observation that 5'-hydroxy chlorsulfuron was also a potent inhibitor of acetolactate synthase (Dr. H. Brown, E.I. du Pont de Nemours & Co., personal communication), the target enzyme of the sulphonylureas, suggests that in this case subsequent glycosidation rather than hydroxylation constitutes the important detoxification step. Sweetser (1985) has speculated on the involvement of an inducible cytochrome P-450 dependent mixed function oxidase (mfo) involved in the hydroxylation of chlorsulfuron in wheat based on the incorporation of one atom of <sup>18</sup>O from molecular oxygen into a hydroxylated metabolite and the sensitivity of chlorsulfuron metabolism to inhibitors of protein biosynthesis.

#### Alkyl hydroxylation

Oxidation of alkyl substituents is also considered to be mediated by microsomal *mfo* enzymes. For both *N*- and *O*-substitutions initial alkyl

hydroxylation results in the formation of alcohol intermediates that are generally unstable and, unless rapidly glycosylated, decompose to liberate formaldehyde in the case of a methyl group. These processes are referred to as N-dealkylation and O-dealkylation respectively. Further oxidation of C-alkyl derived alcoholic functions to corresponding carboxylic acids is generally slow and is probably of little relevance to selectivity.

Hydroxylation of C-alkyl groups is of common occurrence in plants but notable species differences exist which contribute to selectivity. In contrast to graminaceous crops the tolerance of certain broadleaf species to chlorsulfuron results from hydroxylation of the 4-methyl substituent on the triazine ring and subsequent glycosylation rather than 5' ring hydroxylation (Hutchison, Shapiro & Sweetser, 1984). The herbicidal nature of the 4-hydroxymethyl derivative (Sweetser, 1985) implies that for this metabolic route the glycosylation step is also essential for detoxification. A particularly interesting feature of the sulphonylureas is that comparatively subtle modifications to the parent structure can result in targetting for selective use in different crops. Thus DPX-F5384 (Londax) is used for weed control in rice whereas chlorsulfuron is non-selective in this crop (Ray, 1985). Tolerance of rice to DPX-F5384 apparently correlated with an ability to O-demethylate a pyrimidine methoxy substituent to the corresponding hydroxy derivative (Dr. H. Brown, E.I. du Pont de Nemours & Co., personal communication). Differential metabolism also provides an explanation for the selectivity of the imidazolinone aryl-carboxylate herbicides which also inhibit the acetolactate synthase enzyme. Thus Assert, which is comprised of a mixture of m- and p-methyl substituted isomers, was largely de-esterified to the phytotoxic acids in susceptible wild oat whereas ring-methyl hydroxylation to the m- and p-benzyl alcohols followed by glycosylation occurred in tolerant maize and wheat (Brown, Chiu & Miller, 1987).

Selectivity of substituted phenylureas was markedly enhanced by the replacement of the para chlorine of diuron with a methyl group as in chlortoluron which is widely used for weed control in winter wheat. This structural modification offered an additional mode of oxidative attack and extensive ring-methyl hydroxylation was considered to be the basis of the chlortoluron-tolerance of wheat and barley (Ryan *et al.*, 1981; Ryan & Owen, 1982). However the position of the ring methyl substitution can also have a profound effect on selectivity. Thus the chlortoluron isomer, CGA 43057 in which the methyl group is meta located, was non-selective, this being attributed to the poor ability of plants to oxidise the methyl group in this position (Ryan *et al.*, 1981). The margin of selectivity of chlortoluron is inadequate for certain cultivars of wheat and barley. Studies carried out in our own laboratory (Ryan & Owen, 1983) have indicated that detoxification by ring methyl oxidation represented a minor metabolic route in leaf discs of susceptible cultivars in which metabolism was generally slow and restricted to N-mono-dealkylation. This metabolite profile resembled that of susceptible graminaceous weed species. The distinction was less apparent in a similar study using intact plants (Cabanne, Gaillardon & Scalla, 1985) though different cultivars were used. Wheat varieties also show parallel differences in response to the related phenylurea metoxuron and in this case a difference in relative contributions to metabolism of N-demethylation versus O-demethylation of the ring methoxy substituent was the major factor that correlated with varietal reaction (Emami-Saravi, 1979). N-dealkylation also plays a role in the metabolism and selectivity of s-triazines such as atrazine though this pathway is usually of minor significance compared to glutathione conjugation and/or hydrolysis of the C-2 chlorine.

### Sulphoxidation

Sulphide groups or other oxidisable sulphur moieties are of common occurrence in pesticide molecules and have been shown to be sulphoxidised by numerous plant species (Andrawes, Bagley & Herrett, 1971), though the precise mechanism of sulphur-oxidation in plants is not clear. In addition to its role in the bioactivation of various pesticides including herbicides (e.g. EPTC) where species differences can account for selectivity (Casida, Gray & Tilles, 1974), sulphoxidation of alkylsulphide groups is often an important initial step in detoxification. Thus the sulphoxides generated by the oxidation of the methylsulphide ring substituents of terbutryn (Edwards & Owen, unpublished observations) and metribuzin (Frear, Swanson & Mansager, 1985) are substrates for glutathione *S*-transferases which catalyse their conversion to non-phytotoxic peptide conjugates. Subsequent further metabolism of glutathione conjugates can result in the formation of phenylmethylsulphides, as was reported for PCNB (Rusness & Lamoureux, 1980), which are susceptible to sulphoxidation. Thus Blair *et al.* (1984) reported the ability of suspension cultures of various plant species to mediate the enzymic sulphoxidation of the model aromatic alkyl-sulphide *p*-chlorophenylmethyl-sulphide. A relevant observation was that rates of sulphoxidation were greatest in cotton and poorest in tobacco which served to illustrate the existence of species differences with respect to this mode of oxidative attack.

### Hydrolysis

Hydrolysis of carboxylic acid ester groups of various xenobiotics is of widespread occurrence in plants. In the context of herbicides, cleavage of carboxylic esters more commonly results in bioactivation (see e.g. Cole *et al.*, 1987) rather than detoxification and examples of herbicide selectivity attributed to species differences in capacity for ester hydrolysis are discussed elsewhere in this volume. The amide linkage is somewhat similar to that in esters and is attacked in plants by amidases. Amino acid amide conjugates of 2,4-D and 2,4,5-T have been identified for some time as prominent metabolites of these phenoxyalkanoic acid herbicides in some plant species, notably legumes such as soybean (Feung, Hamilton & Mumma, 1973; Arjmand, Hamilton & Mumma, 1978; Scheel & Sandermann, 1981). Of the 20 or so amino acid conjugates that are possible 2,4-D metabolites, those of aspartate and glutamate generally predominate. Studies on the fate of amino acid conjugates of 2,4-D in soybean callus have suggested that these metabolites are readily hydrolysed in plant tissue (Feung *et al.*, 1973; Owen, Hamilton & Mumma, unpublished observation) which probably accounts for their observed biological activity (Feung, Mumma & Hamilton, 1974). The implication from these data is that conversion of 2,4-D to amide conjugates does not constitute an effective detoxification mechanism. Whether plant species differ significantly in ability to hydrolyse amide conjugates has not been extensively investigated though in a screen of the herbicidal properties of 2,4-D amino acid conjugates (Feung, Hamilton & Mumma, 1977) green beans, peas, sunflower and soybean responded similarly.

In contrast, available evidence suggests that arylamide bonds are generally quite resistant to hydrolytic attack in plant tissues. A notable exception is the substituted propionanilide derivative, propanil, which was rapidly hydrolysed in resistant rice plants but not in susceptible weed species such as barnyard grass (Frear & Still, 1968). Hydrolysis of propanil has been attributed to a specific arylacylamidase enzyme (Frear & Still, 1968; Hoagland, 1978) the activity of which was sixty times greater in rice compared to barnyard grass. A more recent report (Oyamada *et al.*, 1986) of hydrolysis of the related naphthoxypropionanilide derivative, naproanilide,

in rice plants suggested that the specificity of the rice arylacylamidase extends beyond propanil. A product of this cleavage reaction was the phytotoxic naphthoxypropionic acid (NOP). Tolerance of rice to naproanilide was attributed to rapid detoxification of NOP by hydroxylation and glycosidation. Amide hydrolysis was also rapid in the susceptible *Sagittaria pygmaea* but unlike rice further metabolism of NOP in this species produced mainly methyl and glucose esters rather than ring hydroxy derivatives. Since the methyl ester was reported to be phytotoxic (Oyamada *et al.*, 1986) and the glucose ester represented a potential source of NOP, this difference in metabolic profile between the two plant species was considered to be a possible mechanism for the herbicidal selectivity of naproanilide.

A further competing detoxification mechanism in the case of the chloro-*s*-triazines is the rapid nucleophilic displacement of the 2-chloro substituent in the triazine ring to give non-phytotoxic 2-hydroxy derivatives. These reactions are not enzymic but are catalysed by the naturally-occurring cyclic hydroxamate, 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine (benzoxazinone, primarily in the roots of tolerant plants such as maize (Hamilton, 1964). Species such as cotton, pea, sorghum and Johnson grass (*Sorghum halepense*) which do not contain benzoxazinone produced insignificant amounts of hydroxy-triazine derivatives. Thus in maize and other grass species the various metabolic options available for detoxification of atrazine and other chlorotriazines include *N*-dealkylation, benzoxazinone-catalysed hydrolysis of the C-2 chlorine or its nucleophilic displacement by glutathione (Jensen, Stephenson & Hunt, 1977). Rapid conversion to hydroxy-atrazine alone confers tolerance in *Coix lachryma-jobi* in which glutathione conjugation plays a very minor role in detoxification. In contrast the ability of susceptible panicoid grasses such as barnyard grass and *Setaria glauca* (yellow foxtail) to carry out any of the above biotransformations was very limited (Jensen *et al.*, 1977).

#### Glutathione conjugation

The role of glutathione *S*-transferases in herbicide detoxification and selectivity has been considered in a number of previous reviews, including Lamoureux & Frear (1979) and Cole *et al.*, (1987). With exceptions, glutathione conjugation products represent the major metabolites in maize and other related panicoid species demonstrating field tolerance to chlorotriazines such as atrazine. Glutathione *S*-transferases are also important in the metabolism of other herbicides including the nitrodiphenylethers fluorodifen (Frear & Swanson, 1973; Diesperger & Sandermann, 1979) acifluorfen (Frear, Swanson & Mansager, 1983) and fomesafen (Evans, Cavell & Hignett, 1987), the chloracetanilides propachlor (Lamoureux, Stafford & Tanaka, 1971), alachlor (Mozer, Tiemeier and Jaworski, 1983) and metolachlor (Edwards & Owen, 1986; Blattman *et al.*, 1986) and the sulphonylurea derivative DPX-F6025 ('Classic') (Dr. H. Brown, E.I. Du Pont de Nemours and Co., personal communication). The latter compound has been developed for broadleaf weed control in tolerant soybean which rapidly detoxifies DPX-F6025 by nucleophilic displacement of the pyrimidine chlorine by homoglutathione. The action of glutathione *S*-transferases on nitrodiphenylethers results in fission of the aromatic ether bond. Activity of the transferase towards fluorodifen was higher in resistant species such as cotton, maize, peanut, pea, soybean and okra than in susceptible cucumber, tomato and marrow (Frear & Swanson, 1973). Thus fluorodifen selectivity appeared to be based on distribution and concentration of the transferase. On purification and further characterisation the glutathione *S*-transferase of pea was found to be very specific for fluorodifen and did not utilise related nitrodiphenylethers such as nitrofen

(Frear & Swanson, 1973). Interestingly the pea enzyme did not utilise atrazine as a substrate and further studies on the metabolism of herbicides which serve as substrates for glutathione S-transferases have established the existence of isoenzymic forms which are specific for particular herbicide types. Thus isoenzyme multiplicity offered a possible explanation for the difference in response to atrazine and EPTC between maize (tolerant to both herbicides) and Panicum miliaceum (proso millet) (atrazine-tolerant only) despite the fact that both herbicides undergo similar routes of detoxification by glutathione conjugation (Ezra & Stephenson, 1986). More recent studies using maize cell suspension cultures suggested that the isoenzymes responsible for the detoxification of the chloracetanilide metolachlor were distinct from the enzyme which conjugated atrazine (Edwards & Owen, 1986).

Once formed tripeptide conjugates are generally broken down in plant tissues. Thus in maize the glutathione conjugate of metolachlor was rapidly converted, by the concerted action of carboxypeptidase and  $\alpha$ -glutamyltranspeptidase enzymes, to the cysteine conjugate which was subsequently oxidised to the thiolactic acid conjugate (Blattman *et al.*, 1986; Owen and Donzel, unpublished observation). Similar pathways have been recorded for the fate of glutathione and homoglutathione conjugates of other herbicides though malonylation of the cysteine conjugates as occurs in fluorodifen metabolism may be of more common occurrence than conversion to thiolactic derivatives.

Though glutathione S-transferases are involved in the transformation of chloracetanilides their role in the selectivity of these herbicides is less certain. However, capacity for peptide conjugation may explain the differing sensitivities of cell suspension cultures of maize and rice to metolachlor in that formation of the glutathione conjugate was some 8-fold faster in maize than in rice (Owen & Donzel, unpublished observation).

In addition to the above parent herbicides the sulphoxide metabolites of dithiocarbamates (EPTC), 2-methylmercapto-s-triazines (e.g. terbutryn) and 3-methylmercapto-as-triazinones (e.g. metribuzin) are also substrates for plant glutathione-S-transferases and species differences in capacity for their conjugation can contribute to selectivity. Detoxification of metribuzin in plants occurs by three competing pathways but a recent report (Frear *et al.*, 1985) suggested that homoglutathione conjugation following sulphoxidation may be the most important route in soybean.

#### Reductive deamination

Though reductive reactions do not generally play a major role in pesticide detoxification by plant tissues the reductive deamination of metribuzin represents an important detoxification mechanism which has been considered to be the basis of the metribuzin-tolerance of some soybean cultivars (Fedtke & Schmidt, 1983). Thus deamination in leaves of tolerant 'Bragg' and 'Tracy M' was considerably more rapid than that observed in susceptible 'Tracy' and 'Coker 156'. The deaminase enzyme activity was apparently located in the peroxisome fraction and required a reduced flavin cofactor under anaerobic ( $N_2$ ) conditions (Fedtke & Schmidt, 1983). A similar enzyme system has been implicated in the detoxification of the related triazinone metamitron in tolerant sugar beet (Fedtke & Schmidt, 1979).

#### Glycosidation

The ability of plants to conjugate acidic and hydroxyl-groups of herbicides and their derivatives is well documented, though the extent of glycosidation may vary between both species and plant tissues. Available

evidence suggests that because of their inherent instability, formation of glucose ester conjugates, even when extensive, does not constitute an effective detoxification mechanism. Nevertheless glycosidation may contribute to detoxification by virtue of the enhanced water solubility of the products which facilitates their disposal in the vacuole. However in the case of chlorsulfuron metabolism in tolerant plant species glycosidation resulted in the conversion of phytotoxic hydroxylated metabolites to inactive glycosides and in suspension cultures of soybean and wheat an O-glucosyl transferase was directly responsible for detoxification of pentachlorophenol (Schmitt *et al.*, 1985). Though less common, formation of N-glycosides also occurs in plants and direct N-glycosidation of chloramben represents a significant detoxification pathway in tolerant species (Frear *et al.*, 1978). N-glycoside formation also offers an additional metabolic route for detoxification of metribuzin. Smith & Wilkinson (1974) presented evidence implicating the importance of N-glycosidation in the tolerance of soybean cultivar 'Bragg' to metribuzin, whereas in susceptible 'Coker 102' and 'Senmes' this was a minor pathway. Intra-species variation of metribuzin N-glycosidation in soybean has also been recorded between tetraploid and diploid lines (Abusteit *et al.*, 1985). There would appear to be little doubt that differential metabolism of metribuzin contributes to selectivity between soybean cultivars but tolerant cultivars may differ with respect to the importance of the three detoxification routes.

#### CONCLUDING REMARKS

Differential metabolism is clearly important in determining selectivity of many herbicides both between species and crop varieties. However, it is important to remember that metabolism may be relevant to selectivity of a particular herbicide in certain crop-weed situations only. In other cases additional factors such as uptake and translocation may be as or more important. Knowledge of the metabolic fate of pesticides in plants might lead to the development of new herbicides which exploit species differences in detoxification. The design of inactive herbicide progenitors which are activated by biotransformation might also result from a study of accumulated metabolism data.

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## THE BIOACTIVATION OF HERBICIDES

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## ABSTRACT

Several herbicidal modes of action are dependent on the metabolic activation of a precursor in the target plant. In such cases the precursor can be regarded as a pro-herbicide. The exploitation of this phenomenon to aid penetration and translocation and to confer selectivity is not as common as that found in the drug or insecticide field but some progress is apparent. Bioactivation reactions include oxidations, reductions and hydrolyses and also the reversible conjugation reactions (e.g. with sugars and amino acids). The rational utilisation of such bioactivation requires a study of the enzymology of xenobiotic metabolism in weeds and cultivars. With this knowledge available, rational chemical modifications can be made to intrinsically reactive molecules so that the herbicidal molecule can be released within the target plant.

## INTRODUCTION

The metabolism of bioactive molecules within target organisms generally effects inactivation (or detoxification). Where the structures of such molecules have been carefully optimised for bioactivity, the profound structural changes imparted by, for example, metabolic hydrolysis or conjugation can be expected to negate the chemistry put into the optimisation process. However, there are several well-known examples of metabolic activation from mode of action studies carried out on a range of drugs and pesticides. That is, the generation or enhancement of bioactivity from an intrinsically inert or weakly active precursor. It is important for a number of reasons to be aware of such processes when they occur. For example, ignorance of a bioactivation step can make nonsense of attempts to construct a structure-activity relationship. Another application of such knowledge is the modification of activity that may be achieved by rationally modulating the bioactivation process. Thus chemical safening and synergism can be achieved by altering rates of bioactivation as well as rates of detoxification. A third and important role for bioactivation is that in selective toxicity. The bioactivation step is another point at which species differences can operate (not always to one's advantage of course, but potentially so).

As more bioactivations are discovered and as we learn more about the biotransformation of xenobiotics generally in plants, the concept of incorporating the requirement for bioactivation is increasingly being investigated. This is an extreme way of modulating bioactivity. The concept is that of the 'pro-bioactive molecule', e.g. the pro-drug, pro-insecticide or pro-herbicide. Application of the concept is at its most advanced in the pharmaceutical field. In the agrochemical field pro-insecticides are better known than pro-herbicides. The former have been

reviewed recently by Drabek & Neumann (1985) in an article that covers our current knowledge. The relative situations with insecticides and herbicides reflects the amounts of information available on the metabolism of the two groups in their target species and in mammals. Most insecticides act on the nervous system and consequently studies on their modes of action (in insects) and toxicity (in mammals) have synergised each other affording reasonably rapid progress. The understanding of the biotransformation of xenobiotics in plants is in a relatively more primitive state. Furthermore, most of the effort put into metabolism studies in plants, particularly by Industry, is directed at the identification of terminal residues. Consequently, initial biotransformations (some of which may be bioactivations) and their products (some of which may be active herbicides) are often ill-defined and sometimes missed completely.

It must be said that most existing pro-pesticides were not rationally developed but were the products of serendipity. However, the concepts are quite old. Sir Rudolph Peters devised the term 'lethal synthesis' in relation to the toxicity of fluorocitric acid in 1953 and Adrien Albert coined the name 'pro-drug' in 1958. A wide range of sophistication is feasible. One can envisage the highly accurate targetting of a toxicant to the active site (analogous to the transport of a cytotoxicant to a tumour on a monoclonal antibody to a tumour antigen). On the other hand, the improved penetration of a bioactive carboxylic acid by presentation as its isopropyl ester is a very simple but very effective approach.

Some established bioactivations of herbicides, classified by reaction type, are described below and, following this, are some examples of potentially exploitable reactions.

#### TYPES OF HERBICIDE BIOACTIVATION

Several types of herbicide alteration can be envisaged. Two have in common the alteration of the herbicide in a way that provides some benefit but such that the active parent molecule can be recovered by metabolism in (or very near) the plant or target within the plant. Thus a functional group of an intrinsically active herbicide can be derivatised to form a new molecule with altered physical properties but which is likely to be a substrate for a known cleavage enzyme in the target plant. An example is the esterification of a carboxylic acid, a reaction that is readily reversed by esterase action. Secondly, an analogue of the herbicide which can be converted back by oxidative, reductive or other processes can be used. A simple example is the use of a primary alcohol which can be oxidised to an active carboxylic acid. Thirdly, if a herbicide is known to act via a bioactivation reaction, there is potential value in synthesising analogues such that the bioactivation rate is enhanced or slowed. For example, the synthesis of an isopropyl ester of a carboxylic acid in place of a methyl ester would slow down the rate of hydrolytic bioactivation, or possibly even change the mechanism to an oxidative process. How these manipulations will affect bioactivity and selectivity cannot be predicted accurately without some reasonably detailed information the xenobiotic enzymology of the target species and on important crop species.

## EXAMPLES OF PRO-HERBICIDE BIOACTIVATION

Oxidative processes $\beta$ -Oxidation

One of the most well-known oxidative bioactivations of a herbicide is that involving the phenoxyalkanoic acids, only the first member of which is intrinsically active. The phenoxyacetic acids (e.g. 2,4-D) are known to mimic the natural auxins (e.g. indolylacetic acid), though their mechanism of action is still not fully understood. They are effectively specific for dicotyledons, grasses being resistant. The higher phenoxyalkanoic acids of appropriate chain lengths are degraded to the herbicidally active phenoxyacetic acids in plants. Any chain containing an odd number of methylene groups affords a pro-herbicide. The chain is degraded by  $\beta$ -oxidation (Wain 1955 a, b). The same happens with the indolylalkanoic acids, with which the effect of chain lengths was first noted (Synerholm & Zimmerman 1947). Compounds containing an even number of methylene groups are oxidised to inactive phenols. The first demonstration of the bioactivation was by Wain in 1955 with 2,4-dichlorophenoxybutyric acid (2,4-DB) which was degraded to 2,4-D in sensitive species. This mechanism introduces additional selectivity (over and above that of 2,4-D) because 2,4-DB is not  $\beta$ -oxidised in legumes and so may be safely used in these crops (Wain 1955 a, b). Modulation of the bioactivation can be achieved by substitution in the benzene ring and this has afforded further selectivity between dicotyledons. Thus MCPB [4-(4-chloro-2-methylphenoxy) butyric acid] undergoes rapid  $\beta$ -oxidation in annual nettles and Canadian thistle but not in clover or celery, providing remarkable selectivity. Slow  $\beta$ -oxidation is not the only cause of tolerance of course. Other processes such as aryl hydroxylation, conjugation (with amino acids and glucose) and decarboxymethylation also play a role in the selectivity of this class of herbicides (Cole 1983). Differential uptake is also thought to be important in cases where 2,4-DB is degraded with equal facility by sensitive and resistant species (Hawf & Behrens 1974). Notwithstanding these other factors, the phenoxyalkanoic acid class represent a classical example of the discovery of a bioactivation and its commercial exploitation.

Aryl hydroxylation

Dichlobenil is hydroxylated at the 3- or 4-position in plants to afford metabolites which are potent uncouplers of oxidative and photosynthetic phosphorylation and inhibitors of photosystem II (Moreland *et al* 1974). Dichlobenil itself has a different mechanism thought to involve the inhibition of the biosynthesis of cellulose. It is not known what relative contribution these phenolic metabolites make to the action of dichlobenil.

N-Dealkylation

Metflurazon, a herbicide which inhibits carotenoid biosynthesis causing bleaching, is of low intrinsic bioactivity. It is bioactivated to norflurazon by N-demethylation (Tantawy *et al* 1984). The unicellular green alga, Chlorella fusca adapts to metflurazon by suppressing its N-

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demethylase activity but it remains susceptible to norflurazon in this condition. The mechanism is supported by findings in higher plants where susceptibility to metflurazon correlates with the accumulation of norflurazon.

N-Demethylation of the N,N-dimethyl-s-triazines, trietazine and ipazine to simazine and atrazine, respectively, increases their activity to photosystem II 100 times (Good 1961).

### S-Oxidation

This is a facile reaction in most life-forms and must be considered in the metabolism of any thioether or S-alkyl-containing xenobiotic. The S-alkyl thiocarbamates (e.g. EPTC) are examples of such. These are soil-applied herbicides used for pre-emergent control. They are active against monocotyledons and various dicotyledons but are mostly used for grass control. They are oxidised in plants and soils (and animals) to their sulphoxides and sulphones. EPTC, for example, forms EPTC sulphoxide, which is more toxic than the parent compound (Casida *et al*, 1974), and the sulphone (which has little activity). These metabolites are carbamoylating agents and may well play a role in the mode of action. However, the situation is complicated by the carbamoylation of glutathione which is the major detoxification reaction (Lay & Casida 1976). Direct injection techniques (Dutka & Komives 1983) recently have shown that the phytotoxicity lies in the order: EPTC sulphone>EPTC>EPTC sulphoxide, the reverse order to that found by Casida *et al* (1974) by application to the roots of germinating oats. Uptake, transport and species differences make it difficult to rationalise these two results, particularly as the precise target of EPTC (or its bioactive metabolite) is unknown. Species differences in the rates of oxidation and glutathione conjugation and the balance between these reactions offer the possibility of selectivity but rational exploitation would seem to be some way off.

Methylmercapto-s-triazine herbicides are also subjected to S-oxidation and glutathione conjugation (Bedford *et al* 1975) but it is not known if the former is a bioactivation.

### Reduction

#### Bipyridylum herbicides

Reductive bioactivation of herbicides appears to be rare but nevertheless exists. The action of the bipyridylum herbicides, diquat and paraquat, is an example. This reduction is one for the purist, involving the addition of an electron. In light the 'quats' are reduced by the electron transport system associated with photosystem I. The subsequent bipyridylum free radical is autoxidised in the presence of oxygen to the original cation with the generation of superoxide anion ( $O_2^-$ ). Phytotoxicity is initiated by OH radicals or by  $H_2O_2$  generated from the superoxide or both (Dodge, 1971).

### Methazole

This compound is a cyclic urea which is inactive but is converted in plants to the photosystem II inhibitor DCPMU (Suzuki & Casida 1981). The reaction probably involves the reductive cleavage of the oxadiazolidinedione ring. It is of interest that the three urea herbicides diuron, linuron and methazole share the common intermediate DCPMU. However, the former two herbicides are intrinsically active.

### Hydrolysis

The concept of improving the penetration into plants of herbicidal carboxylic acids by esterification is one of the simplest yet most effective in current use. The aryl-substituted propionic acid derivatives used for the control of graminaceous weeds (wild oat, etc.) in cereals are rapidly metabolised in the plant to their bioactive, free acid forms. Benzoylprop-ethyl and the related flamprop esters, for example, require de-esterification for activation (Beynon *et al* 1974, Hill *et al* 1978). Benzoylprop acid is much more active as an auxin antagonist than benzoylprop-ethyl. These carboxylic acids act by inhibiting cell elongation and expansion at the stem apices (Jeffcoat & Harries 1973). Precisely how this inhibition is achieved is not known.

The requirement for bioactivation in this series also confers selectivity of action. Jeffcoat *et al* (1977) have postulated that the susceptibility of the wild oat (*Avena fatua*), as compared with the resistant wheat, is due to rapid de-esterification in the weed. Chlorfenprop-methyl is similarly hydrolysed to the free acid in *Avena* (Fedtke & Schmidt 1977) but the rate appears to be similar in weed and cultivar. The pyridinyloxy-phenoxypropionic acid esters are also bioactivated by hydrolysis (Hendley *et al* 1985) as is the imidazolinone aryl-carboxylate herbicide 'Assert' (Brown *et al* 1987). Methyl substitution in the benzene ring of the latter allows rapid oxidative detoxification in corn and wheat.

In some cases there is conflicting evidence for the role of hydrolysis in selectivity. This may be because other pathways operate, e.g. aryl hydroxylation or carboxyl group conjugation.

### Conjugation

Most conjugation processes lead to inactivation. The glutathione conjugation of fluorodifen and atrazine are good and typical examples. However, where the process is reversible, as in the conjugation of carboxylic acid herbicides, the conjugate may well have a transport or a storage role or both. Furthermore, if the conjugation is reversed, e.g. the action of  $\beta$ -glucosidase on a carboxylic glucoside, the conjugate may have a pro-herbicide status.



Amino acid conjugates

Amino acid conjugates of herbicidal acids have biological activity in some cases. This has been checked for a series of conjugates of 2,4-D (Feung et al 1974) and 2,4,5-T (Davidonis et al 1979) using soya bean callus tissue culture (rate of growth) and Avena coleoptile section elongation. The activity has also been studied under field conditions (Feung et al 1977). The more polar conjugates such as 2,4-D-aspartic acid and 2,4-D-glutamic acid were very effective in vitro but poor on whole plants. The less polar conjugates such as 2,4-D-valine and -leucine were strongly herbicidal in the field. Better penetration of the less polar conjugates is the likely explanation. A study of the activity of amino acid conjugates of some other herbicidal carboxylic acids merits some effort.

Glucosides

The glucose ester of diclofop acid accumulates as the major metabolite in susceptible species (Jacobson et al 1985). This is thought to act as a large, readily available pool of diclofop acid. A glucose conjugate would be too polar to penetrate the leaf surface. It may possibly be a candidate for active uptake by root systems but in practice would be too unstable for application to the soil. However, if presented to the plant as a lipophilic, absorbable derivative, pro-herbicidal activity may be possible. It is of interest, therefore, that the tetra-O-acetyl derivative of diclofop glucoside is as inhibitory as diclofop-methyl to the growth of primary roots of oat seedlings (Jacobsen et al 1985).

## MODIFIERS OF HERBICIDAL ACTIVITY

An example of a synergist which owes its activity to bioactivation was described recently. Tridiphane is a herbicide in its own right used for the control of grass and annual broad-leaved weeds in cereals. It also synergises the activity of atrazine in the giant foxtail. The compound will not synergise atrazine in maize nor in the weed when the latter is taller than 10 cm. It is effective, however, in the weed when shorter than 10cm. The mode of action is interesting. Tridiphane is metabolised by glutathione conjugation in both plants (as would be expected from its structure). The conjugate inhibits the conjugation of atrazine with glutathione, presumably by a pseudo 'product' inhibition of glutathione transferase activity. Such inhibition is common for mammalian glutathione transferases. The basis of the selectivity is the rate of catabolism of the inhibiting conjugate in the plants. This is slow in the young giant foxtail, hence atrazine is synergised, but it is rapid in the mature weed and in maize in which the synergist is ineffective (Lamoureux & Rusness 1986).

The inhibition of bioactivation would be expected to afford a safening action. An investigation of the enzymology of the various reactions above could well lead to the discovery or design of effective inhibitors to act as safeners. For example, the carboxylesterases which

bioactivate the numerous carboxylic ester herbicides are obvious candidates. Insecticidal organophosphorus esters, usually effective inhibitors of carboxylesterases, may well serve as safeners via such action.

#### THE DESIGN OF BIOACTIVATED HERBICIDES

The prerequisites for the design of a bioactivated herbicide are firstly, knowledge of the structure of the ultimate phytotoxicant e.g. flamprop acid (as opposed to flamprop-isopropyl) and secondly, the chemical conversion of this toxicant to a derivative which serves as its precursor in the plant. The activation step in the plant will usually be enzyme-catalysed but other mechanisms such as photo-conversion may be effective.

A herbicidal carboxylic acid will serve as a simple example of a range of modifications that could be carried out in the search for a pro-herbicide (Table 1).

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TABLE 1

Some putative pro-herbicides derived from a herbicidal

carboxylic acid RCOOH

Derivative	Structure	Bioactivation reaction(s)
Ester	RCOOR'	hydrolysis
Thioester	RCOSR'	oxidation, hydrolysis
Amide	RCONH <sub>2</sub>	hydrolysis
Nitrile	RCN	hydrolysis (and/or oxidation?)
Alcohol	RCH <sub>2</sub> OH	oxidation (dehydrogenation)
Ether	RCH <sub>2</sub> OCH <sub>2</sub> R'	oxidation (x 2)
Alcohol ester	RCH <sub>2</sub> OCOR'	hydrolysis, oxidation
Alkanoic acid	R(CH <sub>2</sub> ) <sub>n</sub> COOH	$\beta$ -oxidation
Alkanol	R(CH <sub>2</sub> ) <sub>n</sub> CH <sub>2</sub> OH	oxidation, $\beta$ -oxidation
Amino acid conjugate and esters	RCONHCH(R')COOH RCONHCH(R')COOR''	hydrolysis hydrolysis (x 2)
Aldehyde and hemiacetal acetal	RCHO RCH(OH)(OR') RCH(OR') <sub>2</sub>	oxidation hydrolysis, oxidation hydrolysis, oxidation
Schiff base	RCH=NR'	hydrolysis, oxidation
Tetra-acetyl glucoside	RCOO glucAc <sub>4</sub>	hydrolysis (x 5)

Successful design can be frustrated by many variables and so this 'rational' approach currently depends more on luck than on our knowledge of how xenobiotics behave in plants. One of the important variables is the capacity of weeds and cultivars to effect the necessary biotransformations, i.e. the xenobiotic enzymology of plants. Our knowledge is currently insufficient for the design task.

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TRANSFORMATION OF PHENOXYACETIC ACID AND CHLORTOLURON IN WHEAT, BARREN BROME, CLEAVERS AND SPEEDWELL. EFFECTS OF AN INACTIVATOR OF MONOOXYGENASES.

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#### ABSTRACT

In wheat shoots, transformation of phenoxyacetic acid (POA) causes an immediate accumulation of glucoside (O-Glu-POA), whereas levels of free 4-hydroxyphenoxyacetic acid (4-OH-POA) are always small. Aminobenzotriazole (ABT) inhibits degradation of POA by acting specifically on the 4-hydroxylation. A similar mechanism is suggested for inhibition of degradation of chlortoluron in wheat. Moderate sensitivity of cleavers (Galium aparine), barren brome (Bromus sterilis) and tolerance of common field speedwell (Veronica persica) to chlortoluron maybe related to intensive metabolism. In barren brome and cleavers, both ring-methyl hydroxylation and N-demethylations develop but are limited to first oxidations. Speedwell converts chlortoluron by extensive N-demethylations. Metabolic fates of chlortoluron in these weeds are affected differently by ABT. Apparent sensitivities to oxidative reactions to ABT are discussed.

#### INTRODUCTION

A number of oxidative enzyme systems are involved in the early stages of the transformation of phenoxy- and substituted phenylurea herbicides in plants. However, their nature and their activities in different species have received little attention. There is some evidence for the involvement of cytochrome P-450 monooxygenases in N-demethylation of monuron in cotton (Frear, 1968) and of chlortoluron in Jerusalem artichoke (Fonne, 1985), but little has been reported on importance in hydroxylation.

In an attempt to increase our knowledge on the plant enzymes involved in oxidations of herbicides, metabolic effectors whose *in vivo* mode of action is known have been used. Several authors used 1-aminobenzotriazole (ABT), an activated inhibitor of cytochrome P-450 (Ortiz De Montellano and Mathews, 1981). It reduces metabolism of phenoxyacetic acid (POA) in carrot and potato (East, 1984), 4-chloro-1-methylphenoxy acetic acid (MCPA) in potato (Cole and Loughman, 1985) and chlortoluron in wheat (Cabanne *et al.*, 1985a) and in maize and cotton (Cole and Owen, 1987). These effects of ABT have been interpreted as indirect evidence for participation of cyt P-450 monooxygenases in these metabolic systems. However, the pattern of metabolites of chlortoluron in leaves of wheat was such that it was impossible to conclude that effects of ABT were restricted to oxidations and negligible on subsequent conjugations. The simpler metabolism of POA was selected for investigation of the effect of ABT on the 4-hydroxylation separately from the conjugation of this compound in wheat.

The selectivity of chlortoluron in winter cereals is correlated with differences in metabolism between crops and susceptible weeds (Ryan and

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Owen, 1982). Some broadleaf weeds, however, are moderately sensitive to chlortoluron such as cleavers (Galium aparine) or tolerant such as speedwell (Veronica persica). Moreover, brome species, i.e., Bromus diandrus in Spain and barren brome (Bromus sterilis) in France described as tolerant to chlortoluron (Sixto et al., 1984) and isoproturon (Okereke et al., 1981) respectively, have been spreading in cereals in recent years. The second purpose of this study was to determine the fate of chlortoluron in these tolerant weeds and to compare the action of ABT on their oxidative reactions.

### MATERIALS AND METHODS

#### Experiments with phenoxyacetic acid.

Seeds of wheat (Triticum aestivum var. Timmo) were germinated in the dark at 25°C for 2 days. Etiolated seedlings were raised for 2 days in the same conditions in water or ABT solutions at  $10^{-5}$ ,  $10^{-4}$ ,  $10^{-3}$  M. All the solutions (pre-treatments) were adjusted to pH 6. [ $1-^{14}$ C] POA or [ $1-^{14}$ C] 4-OH-POA treatments were applied to tips of excised shoots or roots using an infiltration technique (Hutber et al., 1978). Tips 1 cm long in pairs for shoots ( $1.5 \pm 0.2$  mg d.m.) or by 6 for roots ( $1.1 \pm 0.2$  mg d.m.) received 5  $\mu$ l of labelled substrate (15.6 nmoles). They were infiltrated for 15 or 10 min. for shoots or root, respectively. After incubation samples were crushed onto 3 MM Whatman paper and then chromatographed in n-butanol/ propionic acid/water (2:1.3:1 by vol), followed by radioactive scanning. Each metabolite was expressed as a percentage of the incorporated radioactivity. Experiments have been repeated at least 3 times and each result represents a mean  $\pm$  S.D.

#### Experiments with chlortoluron.

Seeds of wheat, barren brome, cleavers and speedwell were germinated at 17°C for wheat and brome and at 22°C for speedwell. Plants were grown in soil and watered with a nutrient solution.

[Carboxyl  $^{14}$ C] chlortoluron metabolism was studied in the second excised leaf of wheat or brome at the two leaf stage. The whole aerial system was used in the case of cleavers (at the two whorl stage) and speedwell (at the four leaf stage). Leaves and shoots were allowed to absorb CPU with or without  $10^{-4}$  M ABT for 2-4 h. ABT treated plants remained in the presence of ABT for the incubation periods (12 - 72 h). The herbicide and its metabolites were extracted in methanol-water separated by tlc and quantified using a radiochromatogram analyser (Cabanne et al., 1985b). Water-soluble metabolites were submitted to  $\beta$ -glucosidase hydrolysis. The results are the average of two independent experiments ( $\pm$  S.D.).

### RESULTS

#### Experiments with phenoxyacetic acid.

The pathway of POA metabolism in wheat was similar to that found in oat, pea, bean, potato (Hutber et al., 1978; Cole and Loughman, 1982, 1984). Aryl hydroxylation was the major reaction as an intermediate step leading to the subsequent formation of the glucoside O-Glu-POA. An insoluble component ( $R_f = 0$ ) and a minor unidentified metabolite ( $R_f = 0.50$ ) were also formed.

In wheat shoots, POA was quickly metabolised ( $T_{1/2}$  about 1 h) into O-Glu-POA. A transient accumulation of 4-OH-POA reached a maximum of 9.8%

after 1 h and then declined to a level of 3.5%. Levels of the insoluble fraction always remained low (Figure 1A). Conversion of POA was also fast in roots of wheat ( $T_{\frac{1}{2}} < 1$  h) as previously noted (Wilcox *et al.*, 1963). However there was a notable difference between shoot and root in the proportion of metabolites. In roots, 4-OH-POA remained largely free, accounting for 65% of the radioactivity after 1 h of incubation while conjugates did not proceed beyond 21.8% after 4 h. In addition to some traces of the  $R_f = 0.50$  metabolite, the roots incorporated significant quantities of radioactivity into an insoluble component (Figure 1B). Thus the roots represented a suitable material to test the effect of ABT on the 4-hydroxylation of POA. They also provided a convenient means for isolating 4-OH-POA in good yield which could be used to test the effect of ABT on conjugation in shoots.

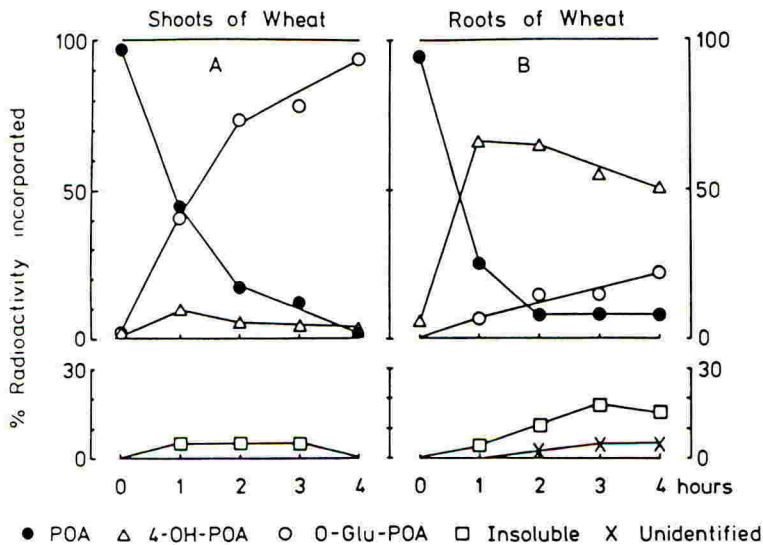


Figure 1. Time-course of POA metabolism in shoots (A) and root (B) of wheat (*T. aestivum* var. Timmo).

ABT had no effect on the growth of shoots and only affected the growth of roots at  $10^{-3}$ M, showing a 34% inhibition (not shown). As expected, metabolism of POA in wheat was sensitive to ABT. Levels of POA and O-Glu-POA in shoots presented an inverse relationship when concentrations of ABT increased (Figure 2A). Again, little 4-OH-POA remained free although a significant level of a compound migrating like 4-OH-POA was noted at the highest rate of ABT. Hydroxylation of POA was inhibited in roots pre-treated with ABT (Figure 2B). An inverse relationship was found on this occasion between POA and 4-OH-POA. Insoluble material and O-Glu-POA only showed a significant decrease at  $10^{-3}$ M ABT.

Rates of conjugation of 4-OH-POA in shoots appeared linear with time and conjugation was complete in about 3 h. After pre-treatments with  $10^{-3}$ M and even higher rates of ABT, formation of O-Glu-POA was not affected (Figure 2C). This result contrasted with the metabolism of POA in shoots and roots. There was little or no conversion of 4-OH-POA into insoluble material in wheat shoots.



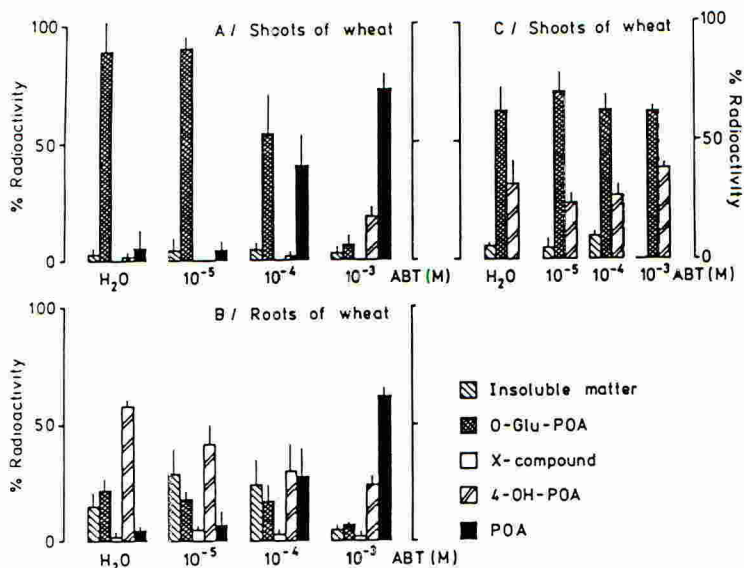


Figure 2. Patterns of metabolites of phenoxyacetic acid, [<sup>14</sup>C] POA, in shoots (A) and roots (B) of wheat pre-treatment with ABT. Accumulation of the glucoside in shoots of wheat (C) pre-treated with ABT and fed with [<sup>14</sup>C] OH-POA. Incubation times were 3 h for A and B and 2 h for C.

#### Experiments with chlortoluron.

Half lives of chlortoluron varied from species to species: wheat = 25 h; barren brome = 14.30 h; cleavers = 10.45 h; speedwell = 6 h (Figure 3). In these weeds degradation of chlortoluron was more rapid than in tolerant wheat. Levels of free-intermediate products never exceeded 5-20%, radio-activity being mainly associated with conjugates. Hydrolysis was carried out when species had converted about 80% of chlortoluron and the value given for each metabolite is the sum of the free and bound forms (Figure 4). Fractions I to IV corresponded to aglycones and the unidentified fraction VI consisted of water-soluble product(s) of hydrolysis not totally converted into aglycones. In all species where this polar product(s) was found its accumulation was always reduced by ABT and it possibly contains residues from both ring-methyl hydroxylation and N-demethylation.

In agreement with results obtained from whole plants (Gross *et al.*, 1979), ring-methyl hydroxylation prevailed in excised leaves of wheat giving nonphytotoxic metabolites. In barren brome and cleavers, both pathways were developed but limited to first oxidations. Speedwell degraded chlortoluron by extensive stepwise N-demethylation. In every species, fractions I to VI responded in a similar way to treatment with ABT. First, metabolism of chlortoluron was reduced, but at different degrees according to species: strongly in wheat, significantly in barren brome and cleavers, weakly in speedwell. Accumulation of hydroxylated metabolites (Fractions IV and V) was inhibited in all species suggesting that ABT was active in all of them and that in these species ring-methyl hydroxylations were always sensitive to the inhibitor. However the inhibition was more marked in wheat than in cleavers.

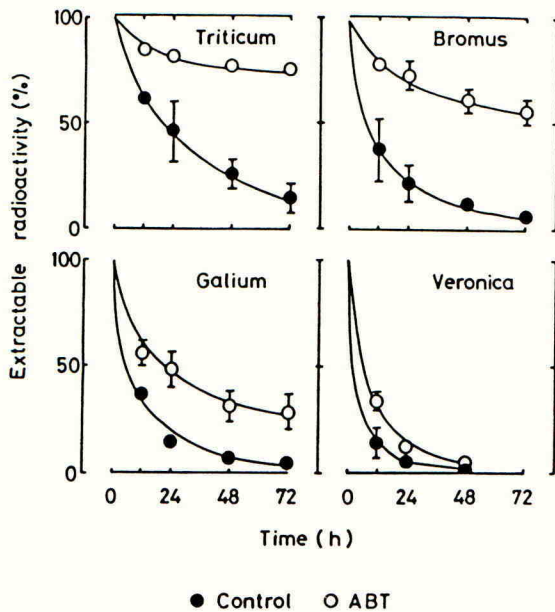


Figure 3. Kinetics of degradation of [ $^{14}\text{C}$ ] chlortoluron in excised leaves of wheat, barren brome and in aerial system of cleavers and speedwell.

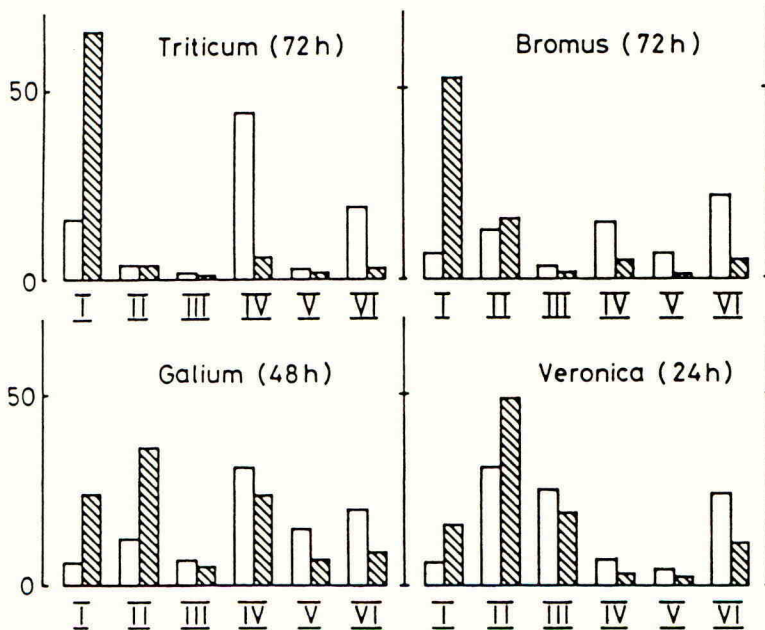


Figure 4. The patterns of metabolism of chlortoluron. Each value is the sum of free and bound forms. I = chlortoluron, II = N-monodemethylated metabolite, III = N-didemethylated metabolite, IV = hydroxymethylphenyl metabolite, V = hydroxymethylphenyl N-monodemethylated metabolite, VI = unidentified fraction

N-monodemethylation was apparently less sensitive to ABT than ring-methyl hydroxylation when applied at  $10^{-4}$ M. Ratios of hydroxylated metabolites (Fractions IV and V) to N-monodemethylated chlortoluron (Fraction II) were modified by ABT. This apparent lower sensitivity of the first N-demethylation with respect to the ring-methyl hydroxylation was not similar for all species, being relatively weak in wheat and barren brome but more significant in cleavers and speedwell. When inhibition of ring-methyl hydroxylation took place, di-N-demethylation never increased, revealing its great sensitivity to ABT.

#### DISCUSSION

Hydroxylation of POA is very effective in both shoots and roots of wheat. This is in agreement with what is known about the capacity of this species to hydroxylate numerous herbicides. Bean, oat, pea are also good hydroxylators but primarily accumulate hydroxylated metabolites (Hutber *et al.*, 1978; Cole and Loughman, 1984), whereas wheat shoots also have substantial capacity for glycosylation. Similar capacities to those of wheat occur in potato (Cole and Loughman, 1982) and in shoots of sorghum but we have not observed them in maize or barley. Quantitative differences between roots and shoots of wheat in the capacity for glycosylation are also apparent.

When ABT is administered to wheat, it inhibits the hydroxylation of POA without affecting its conjugation. A similar effect of ABT on hydroxylation has also been reported in the case of MCPA in potato (Cole and Loughman, 1985). It is possible that the inhibitory effect of ABT on the metabolism of chlortoluron in wheat could also be the consequence of a specific inhibition of oxidative reactions.

Hydroxylated POA is a good precursor of O-Glu-POA in wheat shoots and little insoluble material was formed. The reverse is true for potato, pea and oat (Hutber *et al.*, 1978; Cole and Loughman, 1982) and wheat clearly exhibits a specific characteristic with respect to glucoside formation.

Metabolism of chlortoluron in excised leaves correlated well with what was previously observed in plants. Moderate sensitivities of barren brome, cleavers and speedwell to chlortoluron can be explained by rapid metabolism. In contrast to susceptible weeds, these species are able to conjugate the N-hydroxymethyl intermediate of the first N-demethylation and prevent accumulation of the phytotoxic N-monodemethylated metabolite. Speedwell, in addition, forms the nonphytotoxic N-didemethylated metabolite.

The metabolism of chlortoluron in wheat and the weed species studied was differently affected by ABT, probably reflecting different sensitivities of oxygenases between species, even if other mechanisms can not be excluded. This observation could explain why ABT appeared to be a good synergist of chlortoluron in wheat and not in speedwell (Gaillardon, personal communication). Overall, the hydroxylations studied so far appear particularly sensitive to ABT. Significant sensitivities to ABT have been quoted for hydroxylation of MCPA in potato (Cole and Loughman, 1985) isoproturon in wheat (Cabanne *et al.*, 1985a) and chlortoluron in maize (Cole and Owen, 1987). Nevertheless, hydroxylase(s) present in shoots of cleavers is poorly inhibited by ABT. The second N-demethylation steps are so far sensitive to ABT. A well documented example is that of wheat treated with isoproturon (Cabanne *et al.*, 1985a). In spite of the small number of species studied, it appears that ring-methyl hydroxylation and mono-N-demethylation show

apparent sensitivities to ABT which vary according to species. No discrimination was observed in maize and cotton treated with chlortoluron and ABT (Cole and Owen, 1987) but very small differences could occur in wheat and barren brome and greater differences would be apparent in cleavers and speedwell if our hypothesis about the nature of fraction VI is verified. Further work is needed to characterise the suspected differences in sensitivities of the oxygenases of these weed species to ABT. Their corroboration would be of interest for agricultural applications because of the importance of these differences in the selective action of a number of herbicides.

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## THE FATE OF DIMETHAMETRYN IN THE RAT.

COMPARISON WITH THE METABOLIC PATHWAYS OBSERVED IN PADDY RICE.

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## ABSTRACT

Metabolism of dimethametryn, i.e., N<sup>2</sup>-(1,2-dimethylpropyl)-N<sup>4</sup>-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine, was investigated in rats using [<sup>14</sup>C-U]-labelled material. Major pathways were found to be S-demethylation followed by conjugation with glucuronic acid, N-deethylation, and S-oxide formation followed by conjugation with reduced glutathione. As competitive reactions, hydrolysis of the transient sulfoxide derivatives lead to the formation of minor amounts of ΔC<sup>6</sup>-hydroxy derivatives. N-dealkylation of the dimethylpropyl side chain was found to be less dominant. Metabolic transformation of this residue involved hydroxylation of the methyl groups and subsequently formation of the corresponding carboxylic acid derivatives. Catabolic pathways of the glutathione conjugates in rats and paddy rice are compared.

## INTRODUCTION

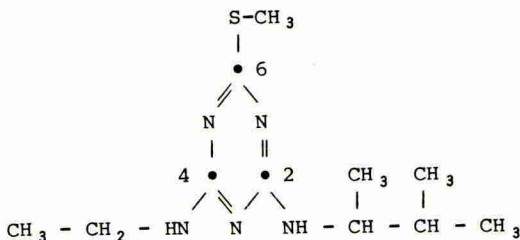


Fig. 1. Dimethametryn.

Dimethametryn (C 18 898), i.e., N<sup>2</sup>-(1,2-dimethylpropyl)-N<sup>4</sup>-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine, is the active ingredient of the herbicide DIMEPAX<sup>®</sup> used for weed control in sugarcane, and one of the active components (together with piperophos) of the herbicide AVIROSAN<sup>®</sup> used in rice. Dimethametryn is slightly toxic to mammals, slightly toxic to birds, and slightly to moderately toxic to fish.

The metabolism of an oral dose of randomly ring <sup>14</sup>C-labelled dimethametryn in the rat is described in this paper. The results are compared with the metabolic pathways observed in paddy rice.

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### MATERIALS AND METHODS

#### Labelled materials

[<sup>14</sup>C-U]-s-triazine labelled dimethametryn with a specific activity of 13.9  $\mu$ Ci/mg and a radiochemical purity >99 % was used in the experiment.

#### Animals and application

Eighteen male rats (strain: Tif: RAI f (SPF)) of about 200 g body weight, kept under controlled conditions (light for 12 h/d, r.h.: 50 - 65 %, temp.: 21 - 24 °C), were orally dosed with <sup>14</sup>C-dimethametryn (ca. 25 mg/kg body weight) dissolved in 1 ml water/polyethylene glycol 200/ethanol 5/2/3 (v/v) by stomach intubation. Urine and faeces were collected at 24 hour intervals during 3 days. Excreta of the first interval were taken for isolation of metabolites.

#### Isolation of metabolites

Metabolites in the urine were extracted first with n-hexane, then with n-hexane/ethylacetate (2/8) and finally with methanol after lyophilization of the water phase. Metabolites of the faeces were extracted with ethylacetate, methanol, and methanol/water (1/1). The apolar metabolite fractions were purified by multiple lc and hplc steps (silicagel columns) or preparative tlc. Water soluble metabolite fractions were purified by reversed phase chromatography (LiChrosorb C<sub>18</sub> or Amberlite XAD-4) or by methylation and/or trimethylsilylation followed by glc purification in the case of acidic and/or desalkylated compounds.

#### Identification of metabolites

Structures of metabolites were determined by ms (ei and ci modes) using a Finnigan Model 4000 instrument. The S-glucuronide and mercapturic acid conjugates were further analyzed by <sup>1</sup>H-nmr on a Bruker HX 360 instrument.

### RESULTS AND DISCUSSION

#### Excretion pattern

Excretion of the radioactivity after oral administration of about 25 mg/kg dimethametryn is summarized in Table 1. One day after administration, about 30 % of the dose was excreted via the kidneys and 33 % were found in the faeces. Three days after administration the excretion values were 38 % and 57 %, respectively. The somewhat delayed excretion of the radioactivity and the high portion excreted in the faeces are partly a consequence of the enterohepatic circulation of glutathione conjugates (Hutson et al., 1970).

TABLE 1

Excretion of radioactivity in rats after oral administration of dimethametryn (~25 mg/kg, n = 18)

Time after application [h]	Excretion (in % of the dose)		
	Urine	Faeces	Total
24	29.9	32.9	62.8
48	7.0	20.9	27.9
72	1.0	2.7	3.7
Subtotal	37.9	56.5	94.4

### Metabolite pattern

The metabolism of dimethametryn in the rat is quite complex. Roughly twenty urinary metabolite fractions and twenty-nine faecal metabolite fractions were observed on tlc one day after application. Unchanged parent was only found in the faeces where it accounted for 1.8 % of the faecal radioactivity. Based on the structures elucidated, the metabolic pathways outlined in Figures 2 to 4 are proposed. The major transformation mechanisms found in general for S-alkyl-s-triazines (Esser, 1975; Larsen, 1978) also operate in the present case. The four initial phase I-reactions appeared to be S-demethylation, N-deethylation, S-oxidation, and demethylpropyl side chain hydroxylation. The formation of transient  $\Delta C^6$ -mercapto groups was evidenced by the presence of reducible disulfide structures in the urine and by the presence of the S- $\beta$ -glucuronide conjugate as major urinary metabolite.

S-oxidation followed by conjugation with reduced glutathione represented a major pathway. The presence of the sulfoxide derivative of parent dimethametryn clearly evidenced this reaction type as activation step for nucleophilic displacement by various nucleophiles like the sulfhydryl group of glutathione or water (Bedford, 1975). Likewise detection of minor amounts of various  $\Delta C^6$ -methoxy derivatives in the methanol extracts reflected reaction with solvent molecules.

Stepwise oxidation of the  $N^2$ -dimethylpropyl side chain and formation of  $N^2, N^4$ -bisdealkylated parent was observed as further, less dominant, degradation route. Hydroxylation occurred at the  $C^1$  and  $C^2$  methyl groups of the side chain. Isolation of a  $C^2 - C^3$  alkene derivative suggested that hydroxylation at the terminal  $C^2$ -methine carbon might also take place; this reaction was observed in rice plants. Further oxidation of the hydroxymethyl derivatives led to the corresponding carboxylic acids.

### Comparison of the conjugation pathways of dimethametryn in rats and paddy rice

The two major phase II-reactions observed in rats were formation of S-glucuronide and glutathione conjugates. Glutathione conjugation was also found as major pathway in paddy rice (Mayer et al., 1980). In contrast to metabolism in rats, it was found to be even more important than N-deethylation, as all major glutathione related metabolites were isolated as  $N^2, N^4$ -bisalkyl derivatives. Catabolism of the glutathione conjugates in the two organisms is shown in Figure 4. The mercapturic acid pathway was found, as expected, in the rat. Identification of the cysteinylglycine conjugate in the faeces confirmed that the first catabolic step involves enzymatic cleavage of the  $\gamma$ -glutamyl residue. The first degradation step in plants was previously shown to involve cleavage of the glycine moiety probably catalyzed by a carboxypeptidase (Lamoureux et al., 1973). The dipeptide is then hydrolyzed to the S-cysteine conjugate which rearranges almost quantitatively in vitro to the N-cysteine derivative. The next step usually involves in the case of triazines, formation of lanthionine conjugates. This transformation was not observed for dimethametryn in paddy rice. Instead, N-dealkylation of the side chain leading to the corresponding  $\Delta C^6$ -amino derivative was observed.



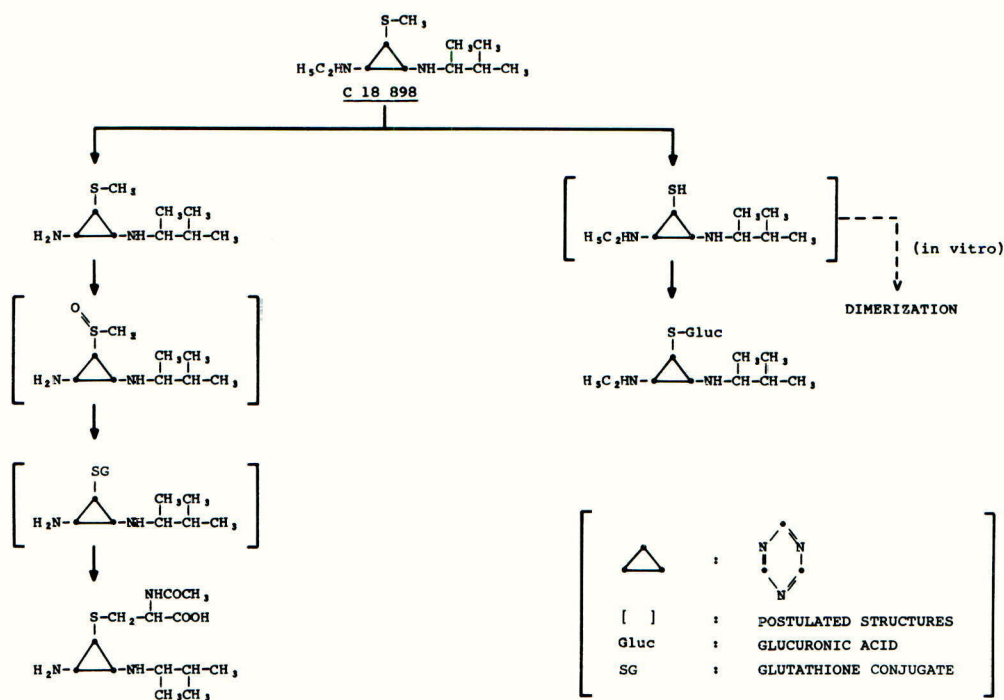


Fig. 2. Major metabolic pathways of dimethametryn observed in the rat.

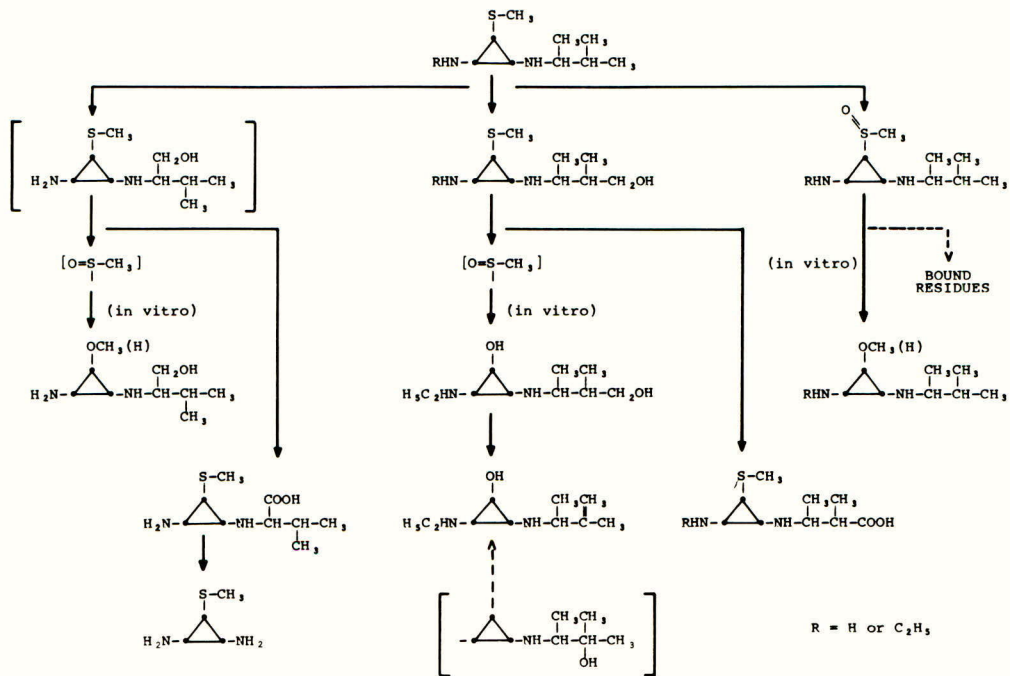


Fig. 3. Minor metabolic pathways of dimethametryn observed in the rat.

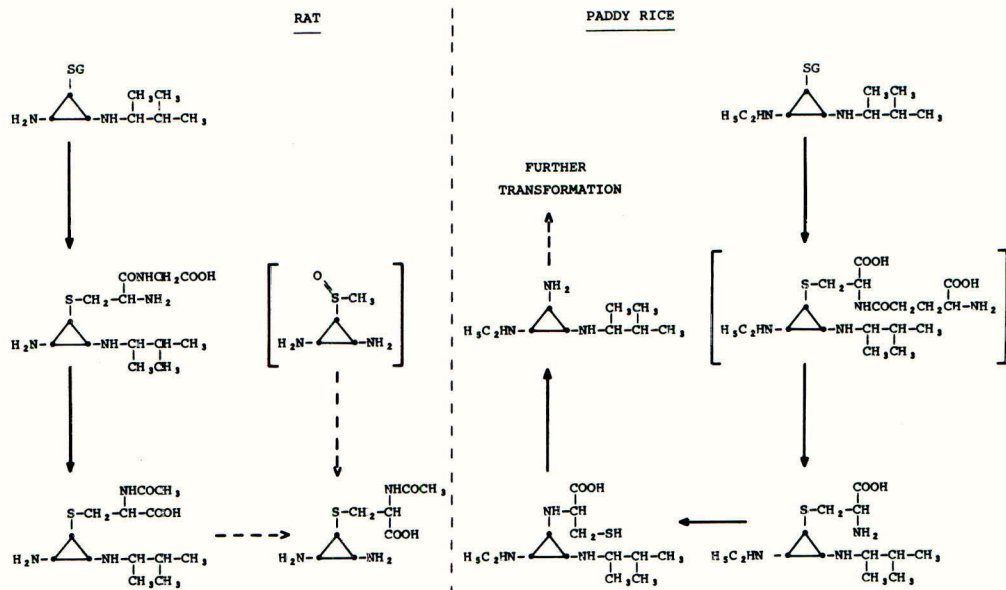


Fig. 4. Catabolic pathways of the glutathione conjugate of dimethametryn in the rat and in paddy rice.

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## FOMESAFEN - METABOLISM AS A BASIS FOR ITS SELECTIVITY IN SOYA

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## ABSTRACT

Soya, maize and spiny cocklebur (*Xanthium spinosum*) plants were treated with  $^{14}\text{C}$ -side-chain labelled fomesafen (Ia) by injection and or by root uptake from nutrient solution. It was established that the facile metabolism of the compound in soya, but not the other species, was the basis for its herbicidal selectivity.

Excised soya leaves were treated with aqueous solutions containing Ia,  $^{14}\text{C}$ -nitrophenyl-labelled fomesafen (Ib) and  $^{14}\text{C}$ -halophenyl-labelled fomesafen (Ic). The same major radioactive metabolite,  $M_1$  was formed following treatments with Ia and Ib but treatment with Ic yielded two different metabolites,  $M_2$  and  $M_3$ . Therefore cleavage at the diphenyl ether linkage was proven. Metabolites  $M_2$  and  $M_3$  hydrolysed with acid to 2-chloro-4-trifluoromethylphenol. Metabolite  $M_1$  was shown by cochromatography to be S-[3-(N-methanesulphonyl-carbamoyl)-4-nitrophenyl]cysteine.

## INTRODUCTION

Fomesafen, (5-(2-chloro-4-trifluoromethylphenoxy)-N-methanesulphonyl-2-nitrobenzamide), 'Flex', is a selective herbicide for use primarily in the control of dicotyledon weeds growing in soya crops. At relatively high treatment levels, grasses may also be controlled. It has been of particular interest to establish the mode of selectivity of fomesafen. This selectivity may be due to any one or combination of the factors listed below.

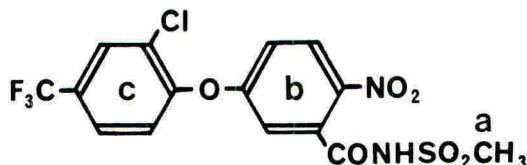
- i) Preferential uptake of fomesafen by either the foliage or the roots of weeds or conversely a specific barrier to uptake in soya plants.
- ii) Selective translocation of the herbicide to an active site in the weed but not in soya.
- iii) Inhibition of a biochemical process common to weed species but absent in soya.
- iv) Conversion of fomesafen to a herbicidally active metabolite in weeds or, conversely, conversion to a herbicidally inactive metabolite in soya.

This paper describes four experiments designed to elucidate the mode of selectivity and metabolism of fomesafen. These experiments involved the uptake of  $^{14}\text{C}$ -fomesafen into shoots of the crop plant, soya (Glycine soy (variety Amsoy)), a monocotyledon weed, maize (Zea mays (variety 3369)) and a dicotyledon weed, spiny cocklebur (Xanthium spinosum).

#### MATERIALS AND METHODS

##### Radiochemicals

Three batches of  $^{14}\text{C}$ -fomesafen were synthesized by the Radiochemical Synthetic Unit, ICI Plant Protection Division, Jealott's Hill Research Station. The positions of the  $^{14}\text{C}$ -radiolabels were as indicated in Figure 1.



- Ia  $^{14}\text{C}$ -side-chain-labelled fomesafen  
 Ib  $^{14}\text{C}$ -nitrophenyl-labelled fomesafen  
 Ic  $^{14}\text{C}$ -halophenyl-labelled fomesafen

Fig. 1. Positions of radiolabelling in  $^{14}\text{C}$ -fomesafen

##### Experiment 1: Hydroponic uptake from nutrient solutions containing $^{14}\text{C}$ -fomesafen (Ia) by soya and maize plants.

Hydroponic uptake solution was prepared by dissolving  $^{14}\text{C}$ -fomesafen (Ia; 31  $\mu\text{Ci}$ ; 950  $\mu\text{g}$ ) into nutrient solution (1 litre; Hoagland and Arnon 1938). Soya and maize plants were grown to a height of 5 cm in sand and the plants were placed in test tubes each containing uptake solution (10 ml). After uptake of all the solution had occurred, non- $^{14}\text{C}$  nutrient solution (10 ml) was added to each tube and this was defined as day 0 of the experiment. Replenishment of tubes with non- $^{14}\text{C}$  nutrient solution was repeated during the course of the experiment. Whole plants were harvested after day 1 and day 14 of the experiment. The roots, rinsed with water (10 ml) and the shoots were analysed separately.

##### Experiment 2: Translocation and metabolism of $^{14}\text{C}$ -fomesafen (Ia) after injection into the stems of soya and cocklebur plants.

Soya and cocklebur plants were grown to a height of between 15 and 20 cm in a bed of sphagnum peat. Each plant was injected with a methanolic solution of  $^{14}\text{C}$ -fomesafen (Ia; 25  $\mu\text{l}$ ; 7  $\mu\text{g}$ ; 0.23  $\mu\text{Ci}$ ) 5 cm above soil level. Whole plants were harvested 1 and 7 days after injection. The whole plants were cut into several sections prior to analysis, ie the injection region (the first 10 cm of plant above the sphagnum peat), the topshoot region (the remainder of the upper plant) and the roots.

Experiment 3: Uptake from solutions of  $^{14}\text{C}$ -fomesafen (Ia, Ib and Ic) and subsequent metabolism by excised soya shoots.

Well-formed trifoliolate leaves were excised from soya plants approximately two months after germination in sphagnum peat. A second cut of each petiole was made under water and the excised leaf was then transferred immediately to a vial containing  $^{14}\text{C}$ -treatment solution ( $^{14}\text{C}$ -fomesafen dissolved in 10 ml of 1% v/v acetone in water; Ia,  $1.19 \times 10^6$  dpm, 16.8  $\mu\text{g}$ ; Ib,  $1.57 \times 10^6$  dpm, 5.8  $\mu\text{g}$ ; Ic,  $0.99 \times 10^6$  dpm, 6.3  $\mu\text{g}$ ). Three vials each containing one leaf were used for each  $^{14}\text{C}$ -treatment. The vials were replenished with distilled water as necessary and all leaves were harvested after 48 hours.

Experiment 4: Hydroponic uptake from nutrient solutions containing  $^{14}\text{C}$ -fomesafen (Ia; Ib and Ic) and subsequent metabolism by soya plants.

Hydroponic uptake solution was prepared by dissolving  $^{14}\text{C}$ -fomesafen (Ib, 5.08  $\mu\text{Ci}$ , 41.2  $\mu\text{g}$ ; dissolved in 100  $\mu\text{l}$  methanol) in nutrient solution (100 ml). A soya plant was grown in sand until the two trifoliolate stage and then transferred to non- $^{14}\text{C}$  nutrient solution. One day later it was transferred to a beaker containing  $^{14}\text{C}$ -uptake solution (25 ml) and was harvested seven days later. The roots and aerial plant parts were analysed separately.

Extraction and quantification of  $^{14}\text{C}$ -residues from plants and shoots.

Any residual treatment or nutrient solutions were assayed by liquid scintillation counting (LSC).  $^{14}\text{C}$ -residues were extracted from all plant tissues by homogenising with methanol followed by filtration. Filtrates were assayed by LSC. Plant debris was air dried and combusted (Packard model 306 sample oxidiser) prior to quantification by LSC.

Characterisation of  $^{14}\text{C}$ -residues in methanol extracts

A generalised scheme of analysis used to characterise the  $^{14}\text{C}$ -residues in methanol extracts from experiments 1 and 2 is shown in Figure 2 and similarly for experiments 3 and 4 in Figure 3. Respective fractions were analysed directly by normal phase thin layer chromatography (TLC, Macherey-Nagel, pre-coated silica gel plates, 0.25 mm layer thickness) using solvent systems as follows.

- Solvent system 1: diethyl ether/hexane/acetic acid 30:20:1 v/v/v
- Solvent system 2: diethyl ether/hexane/acetic acid 18:2:1 v/v/v
- Solvent system 3: propanol/water 2:1 v/v
- Solvent system 4: butanol/water/acetic acid 5:3:2 v/v/v
- Solvent system 5: ethyl acetate/water/formic acid 2:2:1 v/v/v
- Solvent system 6: methanol/chloroform 4:1 v/v
- Solvent system 7: ethyl acetate/formic acid/water 4:1:1 v/v/v
- Solvent system 8: chloroform/ethyl acetate/methanol/acetic acid 12:6:2:1 v/v/v/v

TLC analyses were quantified using a Berthold Automatic TLC Linear Analyser LB2832 with data acquisition, control and analysis system (Le Roy). Cochromatography of authentic reference standards with  $^{14}\text{C}$ -areas was established by autoradiography (Agfa Gevaert Structurix X-Ray film).

## 4B-5

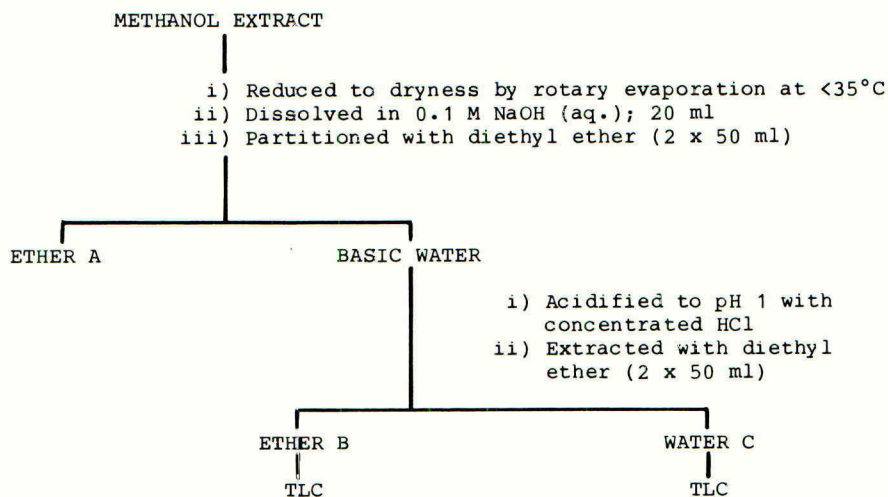


Fig. 2. The generalised fractionation scheme used to characterise  $^{14}\text{C}$ -residues in methanol extracts from experiments 1 and 2.

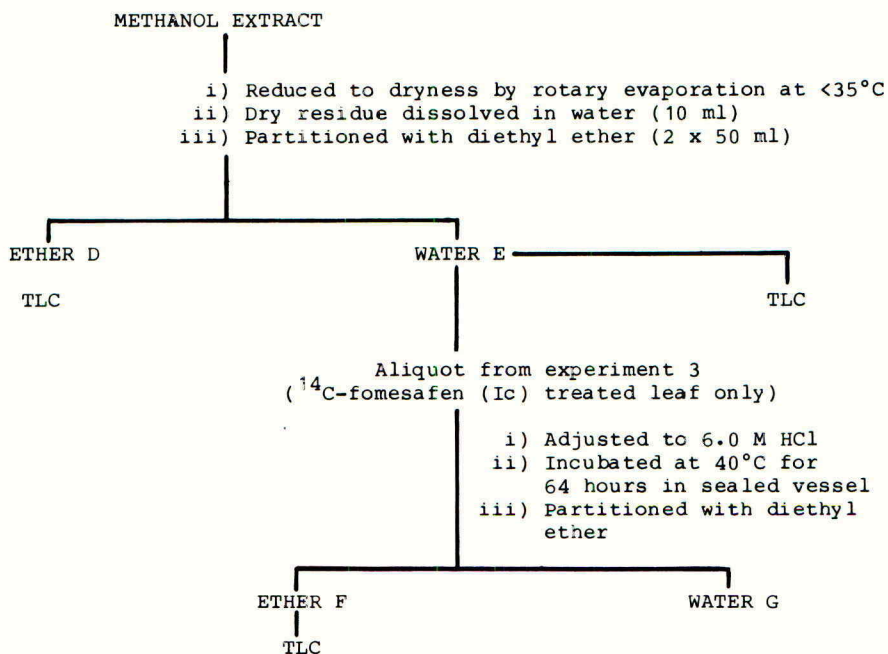


Fig. 3. The generalised fractionation scheme used to characterise  $^{14}\text{C}$ -residues in methanol extracts from experiments 2 and 3.



Gas chromatography/mass spectrometry (GC/MS) was carried out using a VG Micromas 16C mass spectrometer and a Hewlett Packard capillary gas chromatograph linked to a Finnegan Incos data system.

## RESULTS AND DISCUSSION

### Experiment 1

The  $^{14}\text{C}$ -content of root rinsings, residual nutrient solution and methanol extracts were assayed and these results are shown in Table 1. Analysis of methanol extracts according to the scheme shown in Figure 2 resulted in negligible radioactivity partitioning into Ether A fractions. The bulk of the  $^{14}\text{C}$ -residue was distributed between fractions Ether B and Water C. The results from the analyses of the methanol extracts of 1 day shoots are summarised in Table 2.  $^{14}\text{C}$ -uptake into shoots did not appear to be significantly lower in soya than in the susceptible species, maize, (ie there was no appreciably greater root barrier to  $^{14}\text{C}$ -fomesafen in soya). TLC analysis of Ether B fractions using solvent system 1 showed  $^{14}\text{C}$ -fomesafen but no other  $^{14}\text{C}$ -metabolites to be present. Analysis of Water C fractions in the same solvent system showed none of the radioactivity to be mobile and therefore no  $^{14}\text{C}$ -fomesafen was present. These results suggest that metabolism of  $^{14}\text{C}$ -fomesafen (Ia) to polar water soluble  $^{14}\text{C}$ -metabolites was more rapid in the non-susceptible soya plant than in the susceptible weed, maize.

TABLE 1

Radioactivity in fractions from plants previously root treated with  $^{14}\text{C}$ -fomesafen (Ia)

Species	Day	Sample	% of nutrient solution $^{14}\text{C}$ at zero time			
			Extracted into methanol	Not taken up by plant	Remaining in debris	Total recovery
Soya	1	Shoots	8		1	
		Roots	50		4	98
		Root rinsings		1		
		Nutrient		34		
	14	Shoots	6		3	
		Roots	63		8	90
		Root rinsings		1		
		Nutrient		9		
Maize	1	Shoots	5		1	
		Roots	25		4	93
		Root rinsings		5		
		Nutrient		53		
	14	Shoots	10		3	
		Roots	15		7	93
		Root rinsings		4		
		Nutrient		54		

## 4B—5

TABLE 2

The partition of 1 Day shoot extracts

Species	% of original shoot $^{14}\text{C}$ present in:	
	Ether B	Water C
Soya	5	80
Maize	70	31

### Experiment 2

Methanol extracts were analysed according to the fractionation scheme shown in Figure 2 and the results of these analyses are shown in Table 3. TLC analysis of Ether B (solvent system 1) showed  $^{14}\text{C}$ -fomesafen ( $R_f=0.7$ ) to be the only major  $^{14}\text{C}$ -residue, accounting for >75% in most analyses. Similar TLC analyses of Water C fractions showed none of the radioactivity to be mobile and therefore no  $^{14}\text{C}$ -fomesafen was present. The most important results in Table 3 are highlighted. These show clearly that the metabolism was more extensive in the non-susceptible soya plant than in the susceptible weed species cocklebur.

TABLE 3

Radioactivity distribution in fractions from plants previously injected with  $^{14}\text{C}$ -fomesafen (Ia)

Species	Day	Plant Region	% of injected $^{14}\text{C}$ present in Methanol extract	% of methanol extract $^{14}\text{C}$ present in:		% of injected $^{14}\text{C}$ -present as $^{14}\text{C}$ -fomesafen
				Ether B	Water C	
Soya	1	Topshoots	26	8	85	2
		Inj. Region	61	85	4	51
		Roots	1	-	-	-
		Total	88			53
	7	Topshoots	44	3	82	2
		Inj. Region	26	73	30	20
Roots		4	4	86	1	
Total	74	-	23	23		
Cocklebur	1	Topshoots	31	86	4	27
		Inj. Region	62	90	2	56
		Roots	1	-	-	-
		Total	94			83
	7	Topshoots	23	89	2	21
		Inj. Region	51	93	10	47
Roots		<1	-	-	-	
Total	74			68		

## Experiment 3

Greater than 85% of the original treatment radioactivity was taken up into the leaves in each of the experiments. Methanol extracts of the excised leaves were analysed as indicated in Figure 3. The results from these analyses are shown in Table 4.

TABLE 4

Partition of methanol-soluble  $^{14}\text{C}$ -metabolites from excised leaves of soya treated with  $^{14}\text{C}$ -fomesafen

	Radiolabel form of $^{14}\text{C}$ -fomesafen		
	Ib	Ia	Ic
	$^{14}\text{C}$ -content expressed as a percentage of the total radioactivity present in leaves		
Ether D	4	4	13
Water E	62	73	81
Debris	35	28	4

Ether D and Water E were analysed by TLC in solvent system 2.  $^{14}\text{C}$ -fomesafen was present in the ether fractions (<5%) but was absent in the water fractions. Water E was analysed by TLC in the more polar solvent systems 3, 4 and 5. In all three systems the major  $^{14}\text{C}$ -metabolite formed from both labels of  $^{14}\text{C}$ -fomesafen (Ia and Ib) cochromatographed and this compound was referred to as metabolite  $M_1$ . Two major water soluble  $^{14}\text{C}$ -metabolites,  $M_2$  and  $M_3$  were formed from  $^{14}\text{C}$ -fomesafen (Ic). These were significantly less polar than  $M_1$ . The  $^{14}\text{C}$ -metabolite,  $M_1$ , was characterised as, S-[3-(N-methanesulphonylcarbonyl)-4-nitrophenyl]cysteine by cochromatography in four solvent systems (3,4,6 and 7) with an authentic standard. Water E from excised shoots treated with  $^{14}\text{C}$ -fomesafen (Ic) was hydrolysed with 6M HCl as indicated in Figure 3. Virtually all (>85%) of this  $^{14}\text{C}$ -residue partitioned into ether after hydrolysis. Mass spectral confirmation (GC/MS) of the major  $^{14}\text{C}$ -component in Ether F by comparison with that of an authentic sample of 2-chloro-4-trifluoromethyl phenol was achieved.

## Experiment 4

Radioactivity was taken up slowly by soya and translocated to the shoots. After 7 days, 12% of the dose radioactivity was found in the aerial parts of the the plant and of this  $^{14}\text{C}$ -residue, 81% extracted into methanol. The methanol soluble  $^{14}\text{C}$ -residue was partitioned with ether as described in Figure 3. Greater than 90% of the methanol soluble  $^{14}\text{C}$ -residue partitioned into Water E. Water E was analysed by TLC (solvent systems 3 and 4) alongside the corresponding fraction from excised leaves treated with  $^{14}\text{C}$ -fomesafen (Ib; Experiment 3). The major methanol soluble  $^{14}\text{C}$ -metabolite in the aerial parts of the intact plant cochromatographed in both solvent systems with the corresponding methanol soluble  $^{14}\text{C}$ -metabolite,  $M_1$ , in excised leaves.

## CONCLUSIONS

No evidence was found for a larger root barrier to fomesafen in soya than in the susceptible weed species. Fomesafen was shown to be less extensively metabolised in susceptible weeds studied. The tolerance of soya to the herbicide was associated with its more rapid metabolism to less herbicidally active polar metabolites than that of the weed species. The major  $^{14}\text{C}$ -metabolite,  $M_1$ , of  $^{14}\text{C}$ -fomesafen (Ia and Ib) was characterised as S-[3-(N-methanesulphonylcarbamoyl)-4-nitrophenyl]cysteine. Two major  $^{14}\text{C}$ -metabolites,  $M_2$  and  $M_3$ , of  $^{14}\text{C}$ -fomesafen (Ic) were both hydrolysed by acid to yield 2-chloro-4-trifluoromethylphenol, the structure of which was confirmed by GC/MS. Cleavage at the diphenyl ether linkage of  $^{14}\text{C}$ -fomesafen was therefore proven. Similar diphenyl ether cleavage has been reported for another selective substituted diphenyl ether herbicide, acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) in soya plants (Frear et al 1983). A malonyl- $\beta$ -D-glucoside conjugate of 2-chloro-4-trifluoromethylphenol and homogluthathione and cysteine conjugates of the nitrophenyl ring were identified. Although, in our experiments with fomesafen, confirmation of the presence of the corresponding glucoside, malonyl glucoside and homogluthathione conjugate was not undertaken our data is consistent with this pathway. The proposed pathway for fomesafen metabolism in soya is shown in Figure 4.

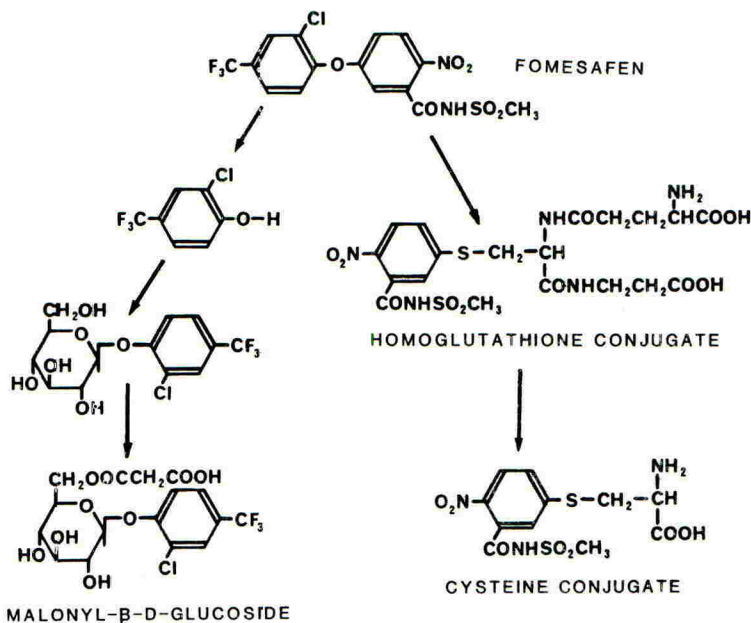


Fig. 4. Metabolism of fomesafen in soya leaves

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## TRICLOPYR : AN INVESTIGATION OF THE BASIS FOR ITS SPECIES-SELECTIVITY

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## ABSTRACT

Radiotracer studies showed that triclopyr ethylene glycol butyl ether ("EB") ester, 2, was taken up more readily than the corresponding solketal ester, 3, or acid form, 1, by wheat, barley, chickweed (Stellaria media) and fat hen (Chenopodium album). Translocation of  $^{14}\text{C}$  following leaf-application of the EB ester was apparently not correlated with tolerance to the ester. Metabolic studies indicated that each species hydrolysed triclopyr EB ester equally efficiently. Extensive metabolism of the acid occurred in each species, but with different metabolite profiles. Triclopyr aspartate was tentatively identified as a major metabolite in chickweed and fat hen plants and soybean cell cultures, whereas wheat and barley metabolised triclopyr predominantly to compounds having the properties of saccharide esters.

## INTRODUCTION

Triclopyr is a post-emergence herbicide used primarily in forestry. It is active against a wide range of dicotyledenous species, although generally inactive against monocotyledenous species. To date, however, no data concerning the fate of triclopyr in plants have been published. The object of the present work was to examine the uptake, translocation and metabolism of triclopyr by a range of representative plant species, and thereby investigate the reason(s) for its selectivity.

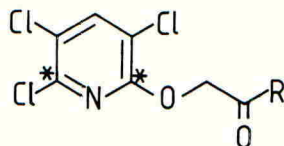
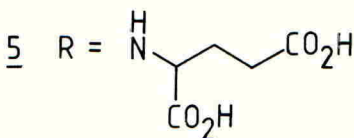
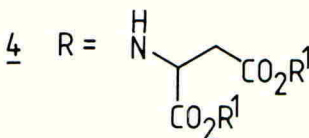
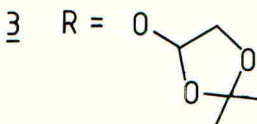
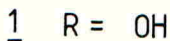
## MATERIALS AND METHODS

Chemicals

Radiolabelled compounds with the following specific radioactivities were used: triclopyr (1) 15.6 mCi/mmol; triclopyr EB ester (2) 13.0 mCi/mmol and triclopyr solketal ester (3) 13.2 mCi/mmol.

Plants and Treatments

The plants used, and their tolerances to triclopyr, were as follows: wheat (cv Norman, tolerant), barley (cv Igri, moderately tolerant), chickweed (Stellaria media, susceptible) and fat hen (Chenopodium album, very susceptible). In addition, a cell suspension culture system of soybean (cv Harcor) was used.

Structural Formulae\* =  $^{14}\text{C}$ 

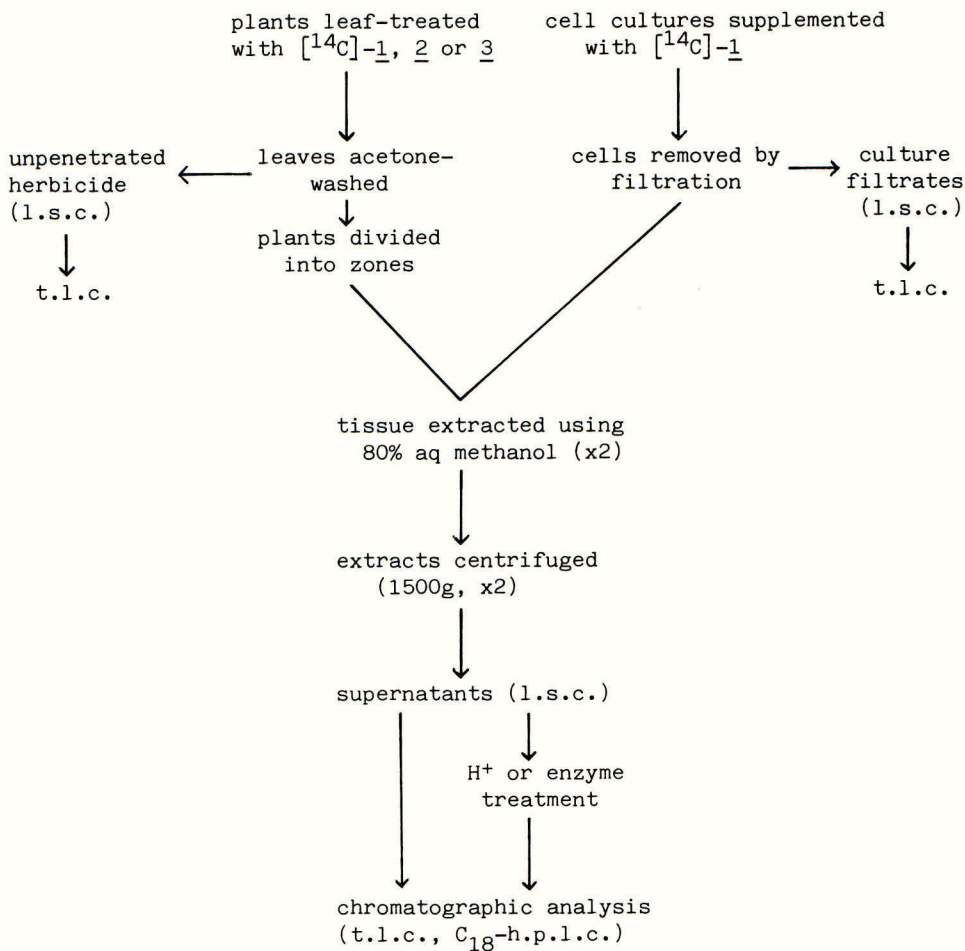
Plants were treated with [ $^{14}\text{C}$ ]-herbicide ( $2 \times 10^6$  d.p.m., equally distributed over a single leaf on each of six plants) in 50% aq acetone + 0.05% Triton X-100. Thirty droplets ( $0.3 \mu\text{l}/\text{droplet}$ ) were applied, via a microsyringe, to the second leaf of wheat or barley at their third leaf stage, or to the fourth leaf of chickweed or fat hen at their sixth leaf stage. Plants were harvested after 3 or 7 days treatment, and divided as follows: wheat, barley - treated zone (A), remainder of treated leaf (B) and rest of plant (C); chickweed and fat hen - treated leaf plus petiole (D) all plant parts above treated leaf petiole (E) and rest of plant (F). Zones were extracted as outlined in Figure 1, and the extracts chromatographed to separate the metabolites present. Experiments with the soybean system used [ $^{14}\text{C}$ ]-tricyclopyr added to the culture medium. The uptake and metabolism of tricyclopyr were determined at intervals as outlined in Figure 1. At these levels of applied herbicide, wheat, barley and soybean cells showed no phytotoxic symptoms, whereas chickweed and fat hen showed epinasty in both petioles and main stems.

Analytical Procedures

After extraction, metabolites were chromatographically compared with synthetic standards using one of two methods. Thin layer chromatography was performed using 0.2 mm thick layers of Kieselgel 60 F<sub>254</sub>, eluting to 15 cm from the origin in acetone-petroleum ether (80-100°)-acetic acid (70:25:0.5 v/v/v). Representative R<sub>F</sub> values were as follows: tricyclopyr EB ester, 0.88; tricyclopyr, 0.69; tricyclopyr aspartate, 0.32. High performance

FIGURE 1

Investigation of the fate of triclopyr and some derivatives in plants and plant cell cultures.



liquid chromatography, using a 250 x 4.9 mm column of 5 $\mu$  Spherisorb C<sub>18</sub>-ODS in series with a 50 x 4.9 mm guard column of CoPell ODS, gave retention times as follows, at 1 ml/min flow rate: triclopyr EB ester, 31.5 min; triclopyr, 22 min; triclopyr aspartate, 13 min. The solvent gradient used was as follows: 0 - 10 min, acetonitrile-water-acetic acid 30:70:0.5 v/v/v; 10 - 30 min: a linear gradient from the initial conditions to 100:0:0.5 and hold. All quantification was done by liquid scintillation counting (l.s.c.).

## RESULTS AND DISCUSSION

Initial whole-plant experiments compared the fate of [<sup>14</sup>C]-triclopyr (1), [<sup>14</sup>C]-triclopyr ethylene glycol butyl ether ("EB") ester, (2) (the derivative used in Garlon 4 formulation) and [<sup>14</sup>C]-triclopyr solketal ester (3). The results indicated that the EB ester was most efficiently taken up by each species, and subsequent detailed metabolism studies concentrated on this compound. Uptake of the EB ester was 94%, or greater, of that applied to each species except chickweed, as summarised in Table 1. The slower rate of uptake might explain the higher tolerance of chickweed, compared with fat hen, to triclopyr.

Radioactivity which penetrated the leaves of these species, when applied as the EB ester, was translocated out of the zone of application as summarised in Table 2. Thus, more radioactivity was translocated in barley than in the other species. This may be a factor contributing to the lower tolerance of barley to triclopyr, when compared with wheat. However, translocation was evidently not of importance in the context of selectivity of triclopyr between the crop plants and the weeds.

Accordingly, attention was focused on the metabolism of triclopyr. In each of the whole plants, hydrolysis of either ester, (2) or (3), was 94% complete, or greater, after 3 days. On the assumption that the true herbicidal species is triclopyr acid (1), activation through de-esterification plays no part in the selectivity of triclopyr in these species. Further, each of the compounds (1, 2 and 3) was extensively metabolised to a range of more polar compounds, as shown in Table 3. The nature of the metabolites was independent of the applied compound but was dependent on the plant species. In particular, wheat and barley metabolised triclopyr to mixtures of polar compounds characterised by their water-solubility ( $R_f = 0$  material, on t.l.c.). Chickweed, fat hen and soybean cell cultures, in contrast, predominantly metabolised triclopyr to compounds intermediate in polarity between the parent molecule (1) and the wheat and barley metabolites. Characteristic of these compounds was their diethyl ether-solubility (at pH 3).

One common property of the metabolites from all species was their acid- and base-catalysed hydrolysis to triclopyr. Thus, all of these plant metabolites are carboxyl derivatives of triclopyr. Similar behaviour has been observed in animal studies (technical data sheet, Dow Chemical Co.), although in soil micro-organisms decarboxylated derivatives have been reported to predominate (Lee *et al.*, 1986).



TABLE 1

Penetration of triclopyr and two esters into wheat, barley, chickweed and fat hen;  $^{14}\text{C}$  expressed as % of that applied.

compound applied	wheat		barley		chickweed		fat hen	
	3d	7d	3d	7d	3d	7d	3d	7d
1	68	68	35	61	61	73	89	83
2	97	99	99	99	58	79	96	94
3	55	90	66	92	50	72	95	95

TABLE 2

Distribution of  $^{14}\text{C}$  after application of [ $^{14}\text{C}$ ]-triclopyr EB ester;  $^{14}\text{C}$  expressed as % of that penetrated into plant; zones as defined in Plants section.

zone	wheat		barley		zone	chickweed		fat hen	
	3d	7d	3d	7d		3d	7d	3d	7d
A	67	64	35	41	D	64	75	68	71
B	28	28	48	39	E	14	12	21	14
C	5	7	17	20	F	21	18	11	15

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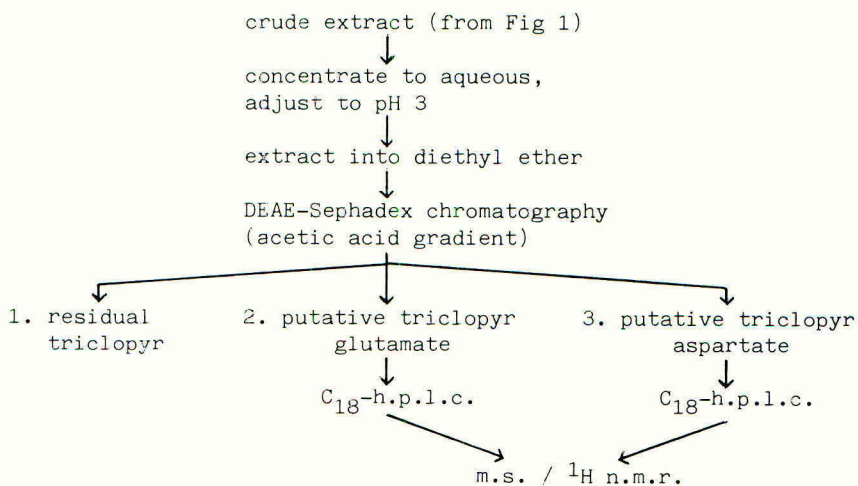
TABLE 3

Distribution of [<sup>14</sup>C]-metabolites formed from triclopyr EB ester in wheat, barley, chickweed, and fat hen. Each metabolite is expressed as % of total, within each column. See text for R<sub>F</sub> values of standards.

R <sub>F</sub>	wheat		barley		chickweed		fat hen	
	3d	7d	3d	7d	3d	7d	3d	7d
0.88	6	2	1	4	3	1	3	2
0.69	9	8	25	22	23	18	26	36
0.40	-	-	2	1	3	1	2	-
0.32	10	7	-	-	65	69	18	14
0.29	-	-	-	-	-	-	2	12
0.22	11	6	-	-	-	-	8	13
0.18	-	-	-	-	-	-	5	-
0.00	65	77	72	73	6	9	35	20

FIGURE 2

Purification of triclopyr metabolites from soybean cell cultures and chickweed plants.



Differential metabolism appeared, therefore, to be potentially important in the species-selectivity of triclopyr. In order to further assess this possibility, investigation of the nature of the major metabolites in each species was required.

Chickweed plants and soybean cell cultures (Lewer and Owen, 1987) metabolised triclopyr to one and two major products, respectively. The physical and chemical properties of these metabolites (acid-catalysed hydrolysis to triclopyr, anion-exchange behaviour, reactivity with diazomethane, solubility in diethyl ether,  $R_F$  on t.l.c.) and literature precedent (Arjmand *et al*, 1978, Mumma and Hamilton, 1978) for metabolism of structurally-related compounds, indicated them to be the aspartate (4,  $R^1 = H$ , major soybean and chickweed) and glutamate (5, minor soybean) amide conjugates. Comparison of the chromatographic properties of the metabolites with those of synthesised standards re-inforced this view (Lewer and Owen, 1987). These metabolites were subsequently isolated using the protocol shown in Figure 2. Structural confirmation (m.s.,  $^1H$  n.m.r.) is currently in progress.

T.l.c. and  $C_{18}$ -h.p.l.c. analysis of the three major fat hen metabolites indicated the most prominent (least polar) one to co-elute with authentic triclopyr aspartate (4,  $R^1 = H$ ). Thus, this amide conjugate appeared to be formed by each of the susceptible species studied. In contrast, barley produced none of this compound, while in wheat less than 10% of the total metabolites co-chromatographed with it. In view of the biological activities of the corresponding 2,4-D- (Feung *et al*, 1974, 1977) and IAA-aspartates (Bialek *et al*, 1983) (correlated, at least in the latter case, with hydrolysis to the parent acid) it was of interest to examine the metabolism and mobility of [ $^{14}C$ ]-triclopyr aspartate (4,  $R^1 = H$ ). This compound was prepared, as its dibenzyl ester to facilitate leaf penetration, by carbodiimide coupling of [ $^{14}C$ ]-triclopyr with dibenzyl aspartate p-toluenesulphonate. [ $^{14}C$ ]-Triclopyr aspartate dibenzyl ester was applied to leaves of both fat hen and chickweed. The results showed that in fat hen, over 7 days, the amide bond of the substrate was cleaved to release triclopyr plus the usual metabolites found in this plant. Too little radioactivity penetrated the leaves of chickweed to give accurate bond hydrolysis data, however very little triclopyr was detected. This observation may account for the relative susceptibilities of the two weeds.

Investigation of the major metabolites in wheat and barley showed them to have the properties of saccharide conjugates (water-solubility, base-lability, glucosidase-catalysed hydrolysis to triclopyr, ion-exchange behaviour). Their ease of chemical hydrolysis to triclopyr suggests that they remain potentially herbicidal, although this remains to be demonstrated *in vivo*. In addition, other phenomena such as sub-cellular compartmentation may play an important role in detoxification. More extensive characterisation of these metabolites, in order to assess their role in the species-selectivity of triclopyr, is currently in progress.

## ACKNOWLEDGEMENT

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