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

β-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 β-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 β-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 β-oxidation in annual nettles and Canadian thistle but not in clover or celery, providing remarkable selectivity. Slow β-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-

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

Bipyridylium herbicides

Reductive bioactivation of herbicides appears to be rare but nevertheless exists. The action of the bipyridylium 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 bipyridylium 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 β-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).

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	$RCONH_2$	hydrolysis
Nitrile	RCN	hydrolysis (and/or oxidation?)
Alcohol	RCH_2OH	oxidation (dehydrogenation)
Ether	RCH_2OCH_2R'	oxidation (x 2)
Alcohol ester	RCH_2OCOR'	hydrolysis, oxidation
Alkanoic acid	$R(CH_2)_nCOOH$	β-oxidation
Alkanol	$R(CH_2)_nCH_2OH$	oxidation, β-oxidation
Amino acid conjugate and esters	$RCONHCH(R')COOH$	hydrolysis
	$RCONHCH(R')COOR''$	hydrolysis (x 2)
Aldehyde and hemiacetal acetal	$RCHO$	oxidation
	$RCH(OH)(OR')$	hydrolysis, oxidation
	$RCH(OR')_2$	hydrolysis, oxidation
Schiff base	$RCH=NR'$	hydrolysis, oxidation
Tetra-acetyl glucoside	$RCOO\ glucAc_4$	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.

REFERENCES

Bedford, C.T.; Crawford, M.J.; Hutson, D.H. (1975) Sulphoxidation of cyanatryn, a 2-mercapto-sym-triazine herbicide, by rat liver microsomes. Chemosphere 4, 311-316.

Beynon, K.I.; Roberts, T.R.; Wright, A.N. (1974) The degradation of the herbicide benzoylprop-ethyl on the foliage of cereal seedlings. Pesticide Biochemistry and Physiology 4, 98-107.

Brown, M.A.; Chiu, T.Y.; Miller, P. (1987) Hydrolytic activation versus oxidative degradation of Assert herbicide, an imidazolinone aryl-carboxylate, in susceptible wild oat versus tolerant corn and wheat. Pesticide Biochemistry and Physiology 27, 24-29.

Casida, J.E.; Gray, R.A.; Tilles, H. (1974) Thiocarbamate sulfoxides: potent, selective and biodegradable herbicides. Science 184, 573-574.

Cole, D. (1983) Oxidation of xenobiotics in plants. In: Progress in Pesticide Biochemistry and Toxicology D.H. Hutson and T.R. Roberts (Eds), Wiley, Chichester, volume 3, pp.199-254.

Davidonis, G.H.; Arjmand, M.; Hamilton, R.H.; Mumma, R.O. (1979) Biological properties of amino acid conjugates of 2,4,5-trichlorophenoxyacetic acid. Journal of Agricultural and Food Chemistry 27, 1086-1088.

Dodge, A. (1971) The mode of action of the bipyridylium herbicides paraquat and diquat. Endeavour 30, 130-135.

Drabek, J.; Neumann, R. (1985) Proinsecticides. In: Progress in Pesticide Biochemistry and Toxicology: Insecticides D.H. Hutson and T.R. Roberts (eds), Wiley, Chichester, volume 5, pp.35-86.

Dutka, F.; Komives, T. (1983) On the mode of action of EPTC and its antidotes. In: Pesticide Chemistry: Human Welfare and the Environment J. Miyamoto and P.C.Kearney (Eds), Pergamon Press, Oxford, volume 3, pp.213-218.

Fedtke, C.; Schmidt, R.R. (1977) Chlorfenprop-methyl: its hydrolysis in vivo and in vitro and a new principle for selective herbicidal action. Weed Research 17, 233-239.

Feung, C-S.; Mumma, R.O.; Hamilton, R.H. (1974) Metabolism of 2,4-dichlorophenoxyacetic acid. VI. Biological properties of amino acid conjugates. Journal of Agricultural and Food Chemistry 22, 307-309.

Feung, C-S.; Hamilton, R.H.; Mumma, R.O. (1977) Metabolism of 2,4-dichlorophenoxyacetic acid. II. Herbicidal properties of amino acid conjugates. Journal of Agricultural and Food Chemistry 25, 898-900.

Good, N.E. (1961) Inhibitors of the Hill Reaction. Plant Physiology 36, 788-803.

Hawf, L.R.; Behrens, R. (1974) Selectivity factors in the response of plants to 2,4-DB. Weed Science 22, 245-249.

Hendley, P.; Dicks, J.W.; Monaco, T.J.; Slyfield, S.M.; Tummon, O.J.; Barrett, J.C. (1985) Translocation and metabolism of pyridinyloxy-phenoxypropionate herbicides in rhizomatous quackgrass (Agropyron repens). Weed Science 33, 11-24.

Hill, B.D.; Stobbe, E.H.; Jones, B.L. (1978) Hydrolysis of the herbicide benzoylprop-ethyl by wild oat esterases. Weed Research 17, 149-154.

Jacobson, A.; Shimabukuro, R.H.; McMichael, C. (1985) Response of wheat and oat seedlings to root-applied diclofop-methyl and 2,4-dichlorophenoxyacetic acid. Pesticide Biochemistry and Physiology 24, 61-67.

Jeffcoat, B.; Harries, W.N. (1973) Selectivity and mode of action of ethyl (±)-2-(N-benzoyl-3,4-dichloroanilino)propionate in the control of Avena fatua in cereals. Pesticide Science 4, 891-899.

Jeffcoat, B.; Harries, W.N.; Thomas, D.B. (1977) Factors affecting the choice of flamprop-methyl, (±)-2-[N-(3-chloro-4-fluorophenyl)benzamido]propionate, for the control of Avena species in wheat. Pesticide Science 8, 1-12.

Lamoureux, G.L.; Rusness, D.G. (1986) Tridiphane [2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane]an atrazine synergist: enzymatic conversion to a potent glutathione S-transferase inhibitor. Pesticide Biochemistry and Physiology 26, 323-342.

Lay, M.M.; Casida, J.E. (1976) Dichloroacetamide antidotes enhance thiocarbamate sulfoxide detoxification by elevating corn root glutathione content and glutathione S-transferase activity. Pesticide Biochemistry and Physiology 6, 442-456.

Moreland, D.E.; Hussey, G.G.; Farmer, F.S. (1974) Comparative effects of dichlobenil and its phenolic alteration products on photo- and oxidative phosphorylation. Pesticide Biochemistry and Physiology 4, 356-364.

Suzuki, T.; Casida, J.E. (1981) Metabolites of diuron, linuron and methazole formed by liver microsomal enzymes and spinach plants. Journal of Agricultural and Food Chemistry 29, 1027-1033.

Synerholm, M.E.; Zimmerman, P.W. (1947) Preparation of a series of -(2,4-dichlorophenoxy) aliphatic compounds and some related compounds with a consideration of their biochemical role as plant growth regulators. Contributions to the Boyce Thompson Institute of Plant Research 14, 369-382.

Tantawy, M.M.; Brauman, T.; Grimme, L.H. (1984) Uptake and metabolism of the phenylpyridazinone herbicide metflurazon during the bleaching and regeneration process of the green alga, Chlorella fusca. Pesticide Biochemistry and Physiology 22, 224-231.

Wain, R.L. (1955a) Herbicidal selectivity through specific action of plants on compounds applied. Journal of Agricultural and Food Chemistry 3, 128-130.

Wain, R.L. (1955b) A new approach to selective weed control. Annals of Applied Biology 42, 151-157.

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

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 ± 0.2 mg d.m.) or by 6 for roots (1.1 ± 0.2 mg d.m.) received 5 µl 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 ± 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 β-glucosidase hydrolysis. The results are the average of two independent experiments (± 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 ($T_{\frac{1}{2}}$ 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.

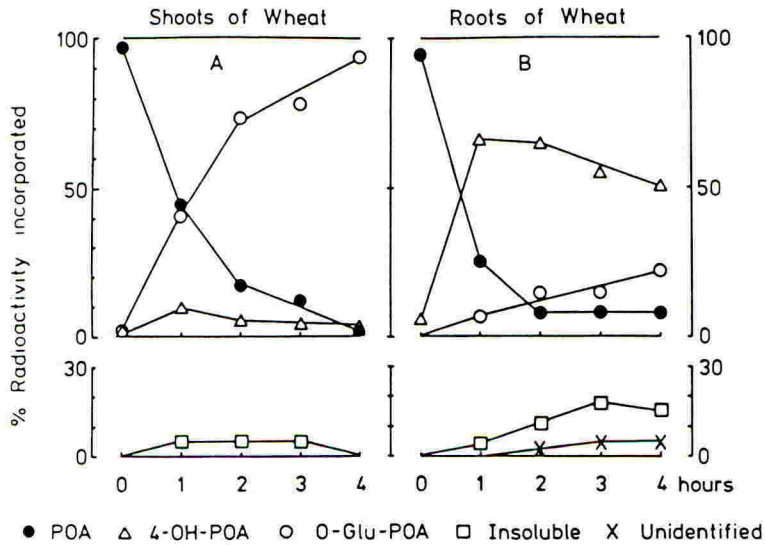

Figure 1. Time-course of POA metabolism in shoots (A) and root (B) of wheat (T. aestivum var. Timmo).

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.

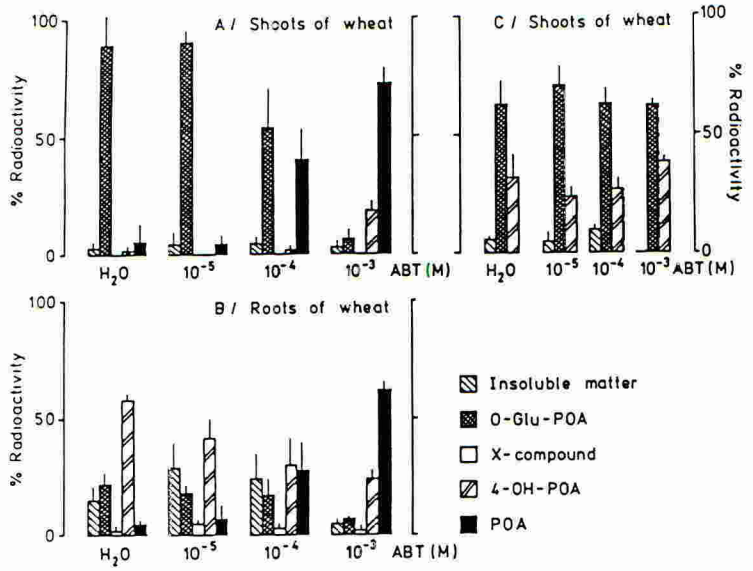

Figure 2. Patterns of metabolites of phenoxyacetic acid, [^{14}C] POA, in shoots (A) and roots (B) of wheat pre-treatment with ABT. Accumulation of the glucoside in shoots of wheat (C) pre-treated with ABT and fed with [^{14}C] OH-POA. Incubation times were 3 h for A and B and 2 h for C.

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.

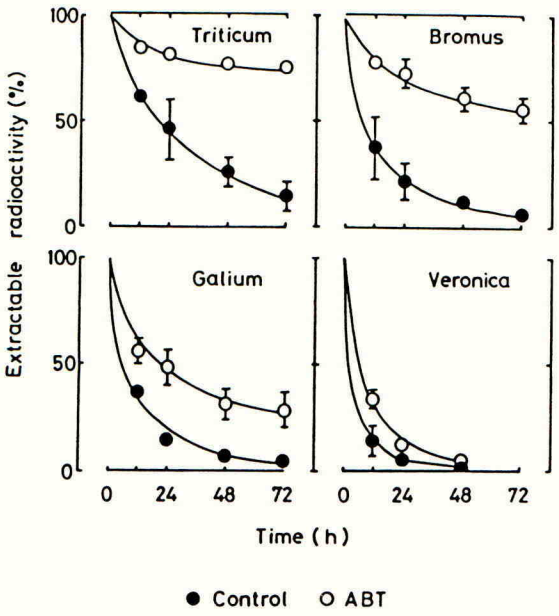

Figure 3. Kinetics of degradation of [^{14}C] chlortoluron in excised leaves of wheat, barren brome and in aerial system of cleavers and speedwell.

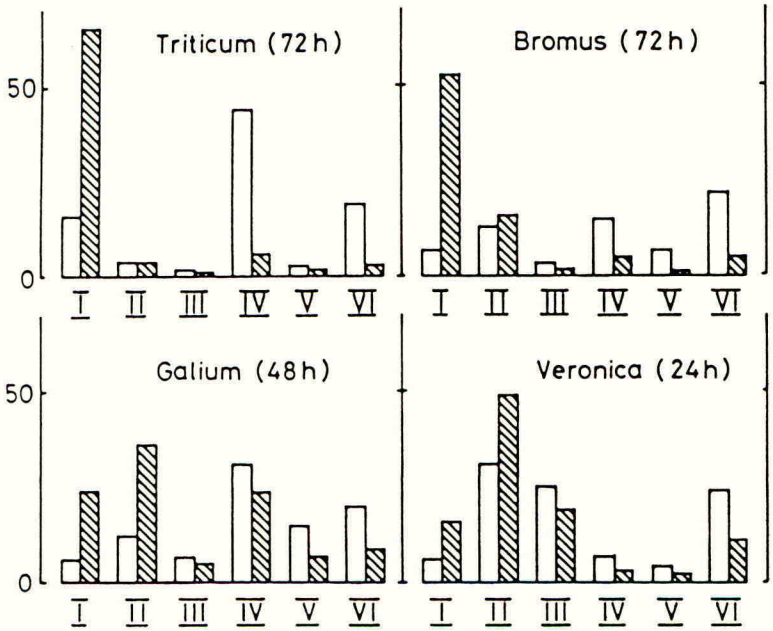

Figure 4. The patterns of metabolism of chlortoluron. Each value is the sum of free and bound forms. I = chlortoluron, II = N-monodemethylated metabolite, III = N-didemethylated metabolite, IV = hydroxymethylphenyl metabolite, V = hydroxymethylphenyl N-monodemthylated metabolite, VI = unidentified fraction

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 sensitivies 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.

REFERENCES

CABANNE, F.; GAILLARDON, P.; SCALLA, R. (1985a). Aminobenzotriazole as a synergist of urea herbicides. Proceedings 1985 British Crop Protection Conference. Weeds 3, 1163-1170.

CABANNE, F.; GAILLARDON, P.; SCALLA, R. (1985b). Phytotoxicity and metabolism of chlortoluron in two wheat varieties. Pesticide Biochemistry and Physiology, 23, 212-220.

COLE, D.J.; LOUGHMAN, B.C. (1982). Metabolism of phenoxyacetic acid in potato (Solanum tuberosum L.) tuber slices. Plant Science Letters, 27, 289-298.

COLE, D. J.; LOUGHMAN, B.C. (1984). Transformation of phenoxyacetic acid herbicides by Phaseolus vulgaris L. callus cells. Plant Cell Reports, 3, 5-7.

COLE, D.J.; LOUGHMAN, B.C. (1985). Factors affecting the hydroxylation and glycosylation of (4-chloro-2-methylphenoxy) acetic acid in Solanum tuberosum tuber tissue. Physiologie Vegetale 23, 879-886.

COLE, D.J.; OWEN, J. (1987). Influence of monooxygenase-inhibitors on the metabolism of the herbicides chlortoluron and metolachlor in cell suspension cultures. Plant Science, 50, 13-30.

EAST, A.K. (1984). Studies on the metabolism of phenoxyacetic acid in carrot and potato. Part II Thesis. Biochemistry. University of Oxford.

FONNE, R. (1985). Intervention du cytochrome P-450 des vegetaux superieurs dans l'oxydation de composes exogenes: l'aminopyrine et le chlortoluron. Thesis Strasbourg.

FREAR, D.S. (1968). Microsomal N-demethylation, by a cotton leaf oxidase system, of 3-(4'-chloro-phenyl)-1,1 dimethylurea (monuron). Science, 162, 674.

GROSS, D.; LAANIO, T.; DUPUIS, G.; ESSER, H.O. (1979). The metabolic behaviour of chlortoluron in wheat and soil. Pesticide Biochemistry and Physiology, 10, 49-59.

HUTBER, G.N.; LORD, E.I.; LOUGHMAN, B.C. (1978). The metabolic fate of phenoxyacetic acid in higher plants. Journal of Experimental Botany, 29, 619-629.

OKEREKE, O.U.; BLAIR, A.M.; CASELEY, J.C. (1981). Some factors effecting the activity of isoproturon against Bromus sterilis and Phalaris minor. Med. Fac. Landbouww. Rijksuniv. Gent, 46, 83-89.

ORTIZ DE MONTELLANO, P.R.; MATHEWS, J.M. (1981). Autocatalytic alkylation of the cytochrome P-450 prosthetic haem group by 1-aminobenzo-triazole. Biochemical Journal, 195, 761-764.

RYAN, P.J.; OWEN, W.J. The mechanism of selectivity of chlortoluron between cereals and grassweeds. In: Proceedings 1982 British Crop Protection Conference. Weeds, 317-324.

SIXTO, H.; GARCIA-BAUDIN, J.M.; CADAHIA, E. (1984). Tolerance de Bromus diandrus Roth. envers differents herbicides antigrominees. In: Proceedings EWRS 3rd Symposium on Weed Problems in the Mediterranean Area, 567-571.

WILCOX, M.; MORELAND, D.E.; KLING-MAN, G.C. (1963). Aryl hydroxylation of phenoxyaliphatic acids by excised roots. Physiologia Plantarum, 16, 565-571.

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^2-(1,2-dimethylpropyl)-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine, was investigated in rats using [^{14}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^6-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

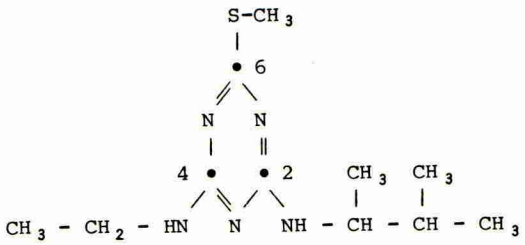

Fig. 1. Dimethametryn.

Dimethametryn (C 18 898), i.e, N^2-(1,2-dimethylpropyl)-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine, is the active ingredient of the herbicide DIMEPAX® used for weed control in sugarcane, and one of the active components (together with piperophos) of the herbicide AVIROSAN® 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 ^{14}C-labelled dimethametryn in the rat is described in this paper. The results are compared with the metabolic pathways observed in paddy rice.

MATERIALS AND METHODS

Labelled materials

[^{14}C-U]-s-triazine labelled dimethametryn with a specific activity of 13.9 µCi/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 ^{14}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_{18} 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 ^{1}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 ΔC^6-mercapto groups was evidenced by the presence of reducible disulfide structures in the urine and by the presence of the S-β-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 ΔC^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 γ-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 ΔC^6-amino derivative was observed.

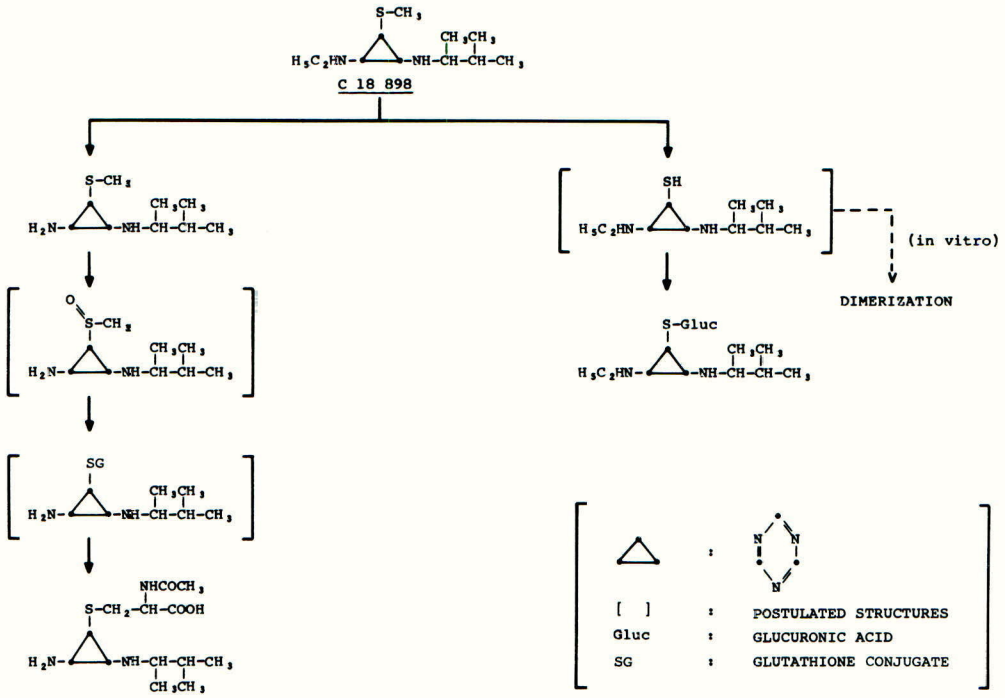

Fig. 2. Major metabolic pathways of dimethametryn observed in the rat.

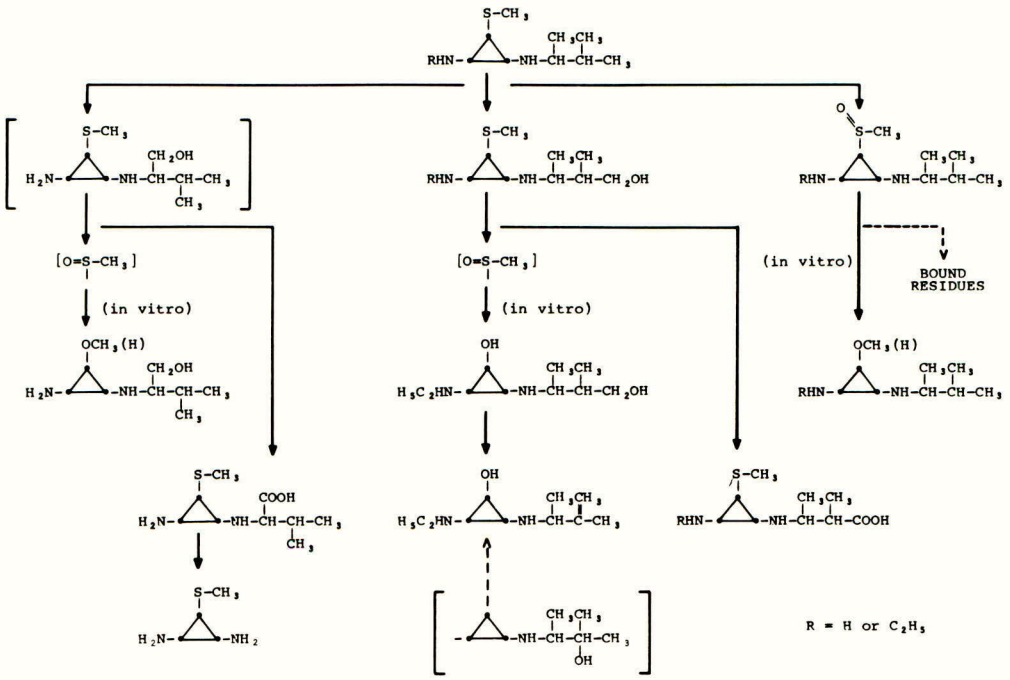

Fig. 3. Minor metabolic pathways of dimethametryn observed in the rat.

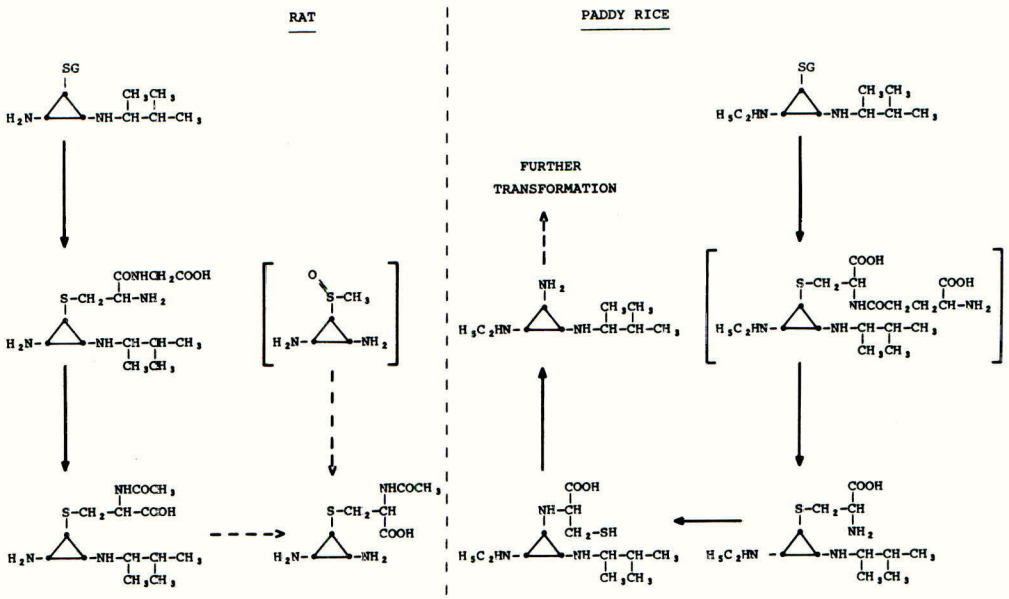

Fig. 4. Catabolic pathways of the glutathione conjugate of dimethametryn in the rat and in paddy rice.

REFERENCES

Bedford, C.T., Crawford, M.J., and Hutson, H. (1975) Sulfoxidation of cyanatryn, a 2-mercapto-sym-triazine herbicide, by rat liver microsomes. Chemosphere 5, 311-316.

Esser, H.O., Dupuis, G., Ebert, E., Marco, G., and Vogel, C. (1975) s-triazines in Herbicides, Chemistry, Degradation and Mode of Action, Vol. 1, 2nd ed. by P.C. Kearney and D.D. Kaufman, Marcel Dekker, New York, pp. 129-208.

Hutson, D.H., Hoadley, E.C., Griffiths, M.H., and Donninger, C. (1970) Mercapturic acid formation in the metabolism of 2-chloro-4-ethylamino-6-(1-methyl-1-cyanoethylamino)-s-triazine in the rat. Journal of Agricultural and Food Chemistry 18 (3), 507-512.

Lamoureux, G.L., Stafford, L.E., Shimabukuro, R.H., and Zaylskie, R.G. (1973) Atrazine metabolism in sorghum: Catabolism of the glutathione conjugate of atrazine. Journal of Agricultural and Food Chemistry 21 (6), 1020-1030.

Larsen, G.L., Bakke, J.E., and Feil, V.J. (1978) Metabolism of [^{14}C]terbutryn {2-(t-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine} by rats and goat. Biomedical mass spectrometry 5 (6), 382-390.

Mayer, P., Kriemler, H.-P, and Laanio, T.L. (1980) Metabolism of N-(1',2'-dimethylpropyl)-N'-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine in paddy rice. Agricultural and Biological Chemistry 45 (2), 361-368.

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}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}C-nitrophenyl-labelled fomesafen (Ib) and ^{14}C-halophenyl-labelled fomsafen (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.

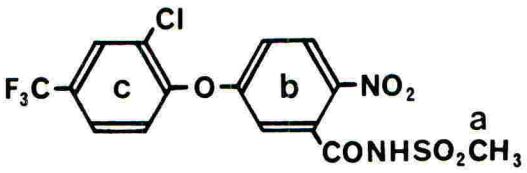
iv) Conversion of fomesafen to a herbicidally active metabolite in weeds or, conversely, conversion to a herbicidally inactive metabolite in soya.

This paper describes four experiments designed to elucidate the mode of selectivity and metabolism of fomesafen. These experiments involved the uptake of ^{14}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}C-fomesafen were synthesized by the Radiochemical Synthetic Unit, ICI Plant Protection Division, Jealott's Hill Research Station. The positions of the ^{14}C-radiolabels were as indicated in Figure 1.



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

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

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

Hydroponic uptake solution was prepared by dissolving ^{14}C-fomesafen (Ia; 31 µCi; 950 µg) 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}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}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}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}C-fomesafen (Ia; 25 µl; 7 µg; 0.23 µCi) 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}C-fomesafen (Ia, Ib and Ic) and subsequent metabolism by excised soya shoots.

Well-formed trifoliate 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}C-treatment solution (^{14}C-fomesafen dissolved in 10 ml of 1% v/v acetone in water; Ia, 1.19 x 10^6 dpm, 16.8 µg; Ib, 1.57 x 10^6 dpm, 5.8 µg; Ic, 0.99 x 10^6 dpm, 6.3 µg). Three vials each containing one leaf were used for each ^{14}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}C-fomesafen (Ia; Ib and Ic) and subsequent metabolism by soya plants.

Hydroponic uptake solution was prepared by dissolving ^{14}C-fomesafen (Ib, 5.08 µCi, 41.2 µg; dissolved in 100 µl methanol) in nutrient solution (100 ml). A soya plant was grown in sand until the two trifoliate stage and then transferred to non-^{14}C nutrient solution. One day later it was transferred to a beaker containing ^{14}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}C-residues from plants and shoots.

Any residual treatment or nutrient solutions were assayed by liquid scintillation counting (LSC). ^{14}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}C-residues in methanol extracts

A generalised scheme of analysis used to characterise the ^{14}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: ehtyl 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 aquisition, control and analysis system (Le Croy). Cochromatography of authentic reference standards with ^{14}C-areas was established by autoradiography (Agfa Gevaert Structurix X-Ray film).

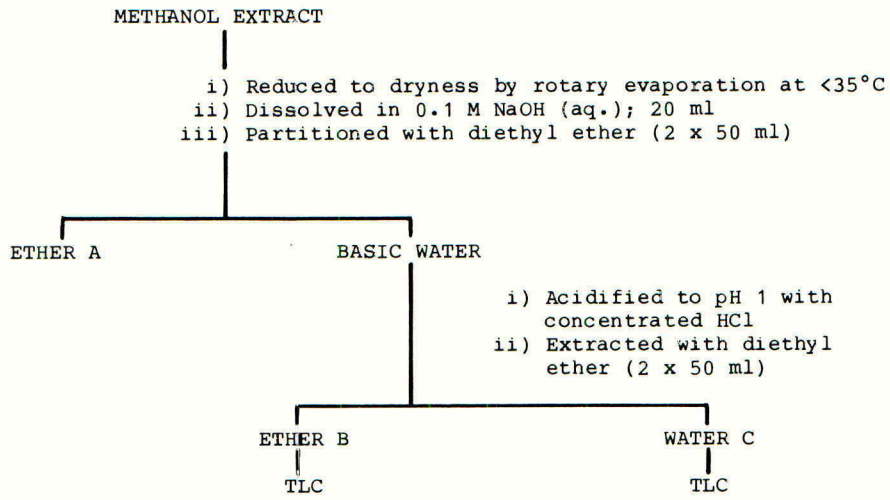


Fig. 2. The generalised fractionation scheme used to characterise ^{14}C-residues in methanol extracts from experiments 1 and 2.

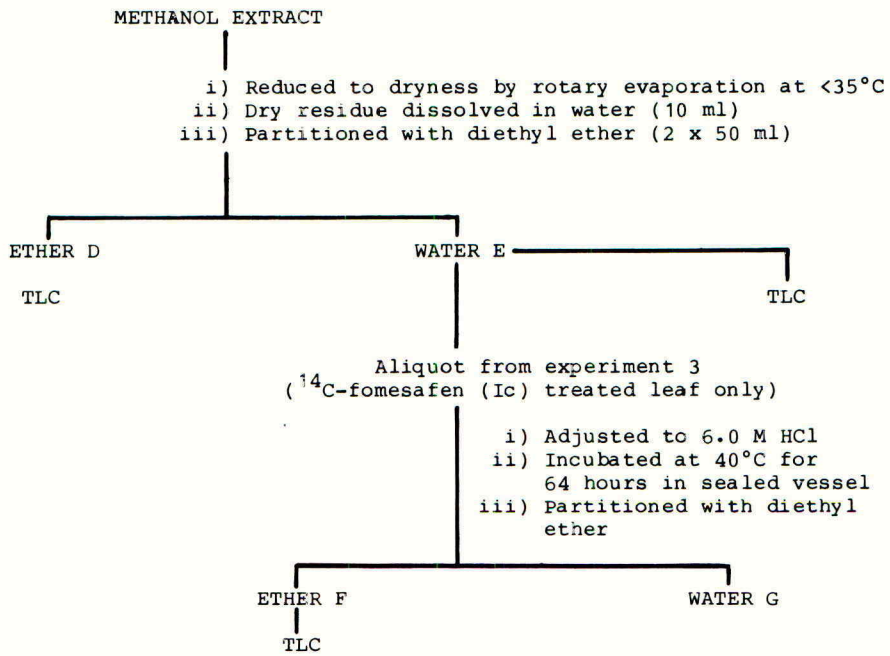


Fig. 3. The generalised fractionation scheme used to characterise ^{14}C-residues in methanol extracts from experiments 2 and 3.

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}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}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}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}C-fomesafen in soya). TLC analysis of Ether B fractions using solvent system 1 showed ^{14}C-fomesafen but no other ^{14}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}C-fomesafen was present. These results suggest that metabolism of ^{14}C-fomesafen (Ia) to polar water soluble ^{14}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}C-fomesafen (Ia)

Species	Day	Sample	% of nutrient solution ^{14}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		

TABLE 2

The partition of 1 Day shoot extracts

Species	% of original shoot ^{14}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}C-fomesafen (R_f=0.7) to be the only major ^{14}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}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}C-fomesafen (Ia)

Species	Day	Plant Region	% of injected ^{14}C present in Methanol extract	% of methanol extract ^{14}C present in :		% of injected ^{14}C-present as ^{14}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}C-metabolites from excised leaves of soya treated with ^{14}C-fomesafen

	Radiolabel form of ^{14}C-fomesafen		
	Ib	Ia	Ic
	^{14}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}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}C-metabolite formed from both labels of ^{14}C-fomesafen (Ia and Ib) cochromatographed and this compound was referred to as metabolite M_1. Two major water soluble ^{14}C-metabolites, M_2 and M_3 were formed from ^{14}C-fomesafen (Ic). These were significantly less polar than M_1. The ^{14}C-metabolite, M_1, was characterised as, S-[3-(N-methanesulphonylcarbamoyl)-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}C-fomesafen (Ic) was hydrolysed with 6M HCl as indicated in Figure 3. Virtually all (>85%) of this ^{14}C-residue partitioned into ether after hydrolysis. Mass spectral confirmation (GC/MS) of the major ^{14}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}C-residue, 81% extracted into methanol. The methanol soluble ^{14}C-residue was partitioned with ether as described in Figure 3. Greater than 90% of the methanol soluble ^{14}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}C-fomesafen (Ib; Experiment 3). The major methanol soluble ^{14}C-metabolite in the aerial parts of the intact plant cochromatographed in both solvent systems with the corresponding methanol soluble ^{14}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}C-metabolite, M_1, of ^{14}C-fomesafen (Ia and Ib) was characterised as S-[3-(N-methanesulphonylcarbamoyl)-4-nitrophenyl]cysteine. Two major ^{14}C-metabolites, M_2 and M_3, of ^{14}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}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-β-D-glucoside conjugate of 2-chloro-4-trifluoromethylphenol and homoglutathione 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 homoglutathione conjugate was not undertaken our data is consistent with this pathway. The proposed pathway for fomesafen metabolism in soya is shown in Figure 4.

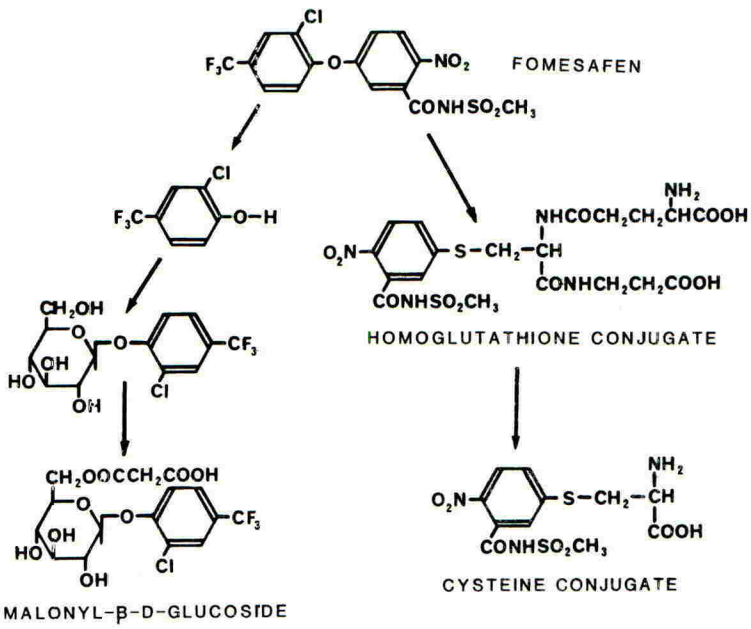


Fig. 4. Metabolism of fomesafen in soya leaves

REFERENCES

Hoagland, D.R. and Arnon, I.R. (1983) California Agricultural Experimental Station Circular, 347, 1-39.

Frear D.S.; Swanson, H.R. and Mansager, E.R., (1983) Pesticide Biochemistry and Physiology, 20, 299.

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}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

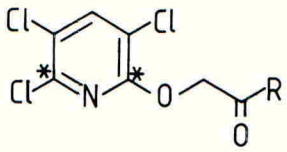
Chemicals

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.

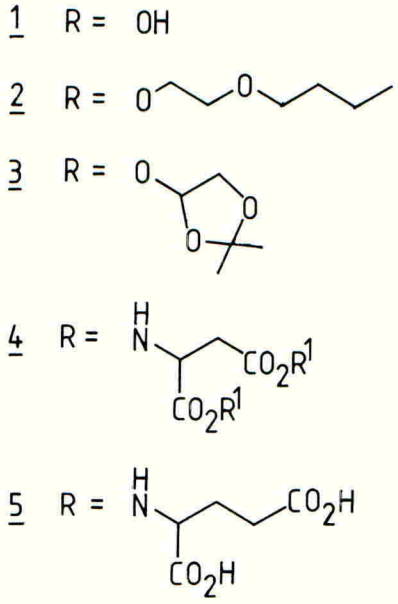
Plants and Treatments

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}C



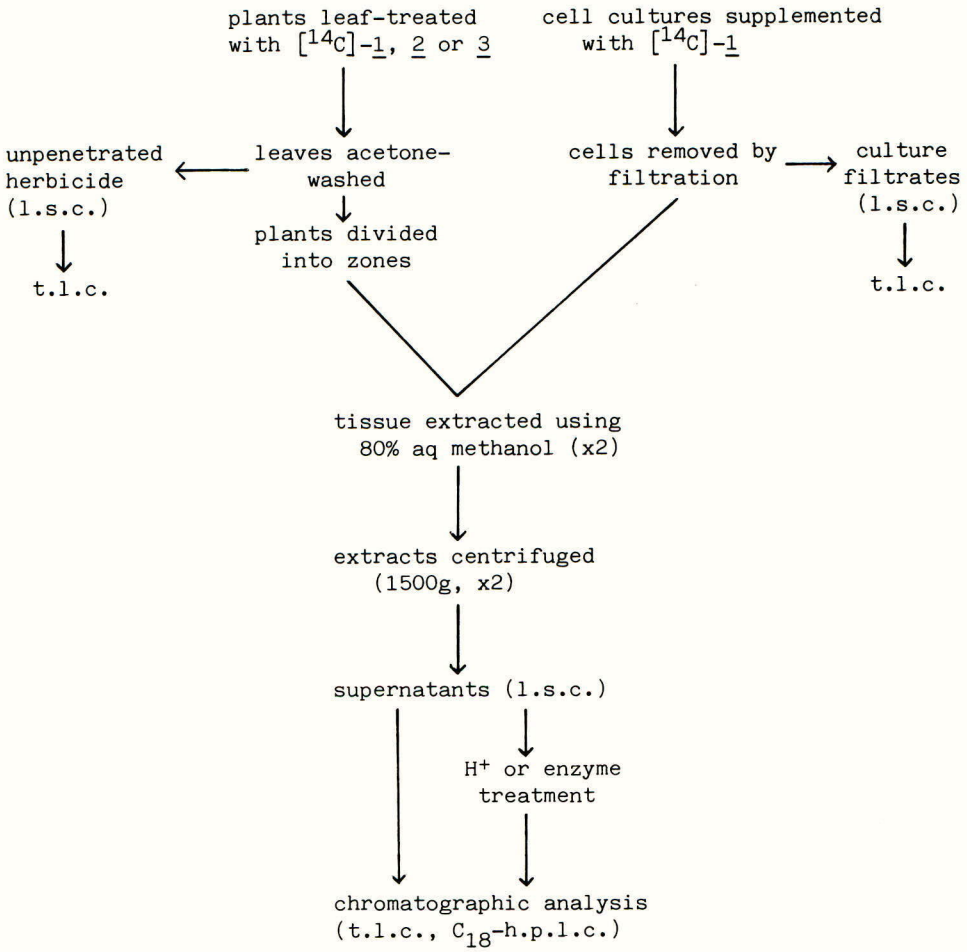
Plants were treated with [^{14}C]-herbicide (2 x 10^6 d.p.m., equally distributed over a single leaf on each of six plants) in 50% aq acetone + 0.05% Triton X-100. Thirty droplets (0.3 µl/droplet) were applied, via a microsyringe, to the second leaf of wheat or barley at their third leaf stage, or to 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}C]-triclopyr added to the culture medium. The uptake and metabolism of triclopyr 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_{254}, eluting to 15 cm from the origin in acetone-petroleum ether (80-100°)-acetic acid (70:25:0.5 v/v/v). Representative R_F values were as follows: triclopyr EB ester, 0.88; triclopyr, 0.69; triclopyr 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 5μ Spherisorb C_{18}-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 [^{14}C]-triclopyr (1), [^{14}C]-triclopyr ethylene glycol butyl ether ("EB") ester, (2) (the derivative used in Garlon 4 formulation) and [^{14}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}C expressed as % of that applied.

compound applied	wheat 3d	wheat 7d	barley 3d	barley 7d	chickweed 3d	chickweed 7d	fat hen 3d	fat hen 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}C after application of [^{14}C]-triclopyr EB ester; ^{14}C expressed as % of that penetrated into plant; zones as defined in Plants section.

zone	wheat 3d	wheat 7d	barley 3d	barley 7d
A	67	64	35	41
B	28	28	48	39
C	5	7	17	20

zone	chickweed 3d	chickweed 7d	fat hen 3d	fat hen 7d
D	64	75	68	71
E	14	12	21	14
F	21	18	11	15

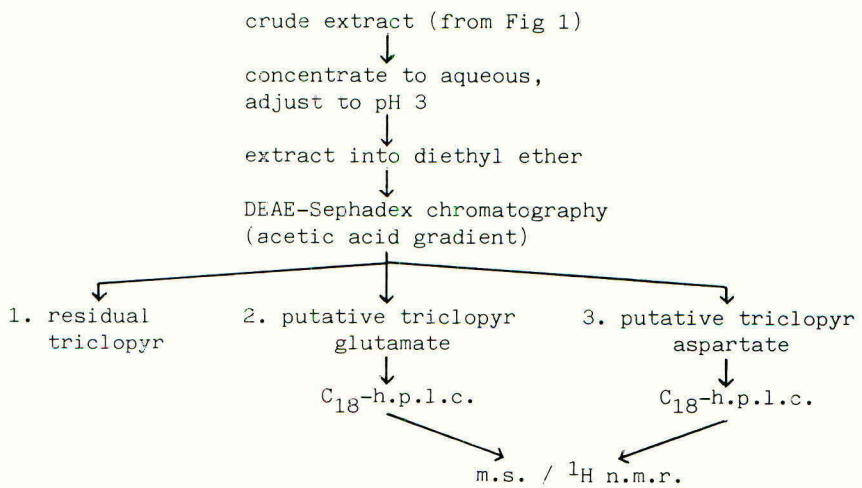
TABLE 3

Distribution of [^{14}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_F values of standards.

R_F	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., ^{1}H 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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REFERENCES

Arjmand, M., Hamilton, R.H. and Mumma, R.O. (1978) Metabolism of 2,4,5-Trichlorophenoxyacetic Acid. Evidence for Amino Acid Conjugates in Soybean Callus Tissue, Journal of Agricultural and Food Chemistry, 26, 1125-1128.

Bialek, K., Meudt, W.J. and Cohen, J.D. (1983) Indole-3-acetic Acid (IAA) and IAA Conjugates Applied to Bean Stem Sections: IAA Content and the Growth Response, Plant Physiology, 73, 130-134.

Feung, C-S., Mumma, R.O. and Hamilton, R.H. (1974) Metabolism of 2,4-Dichlorophenoxyacetic Acid. VI. Biological Properties of Amino Acid Conjugates, Journal of Agricultural and Food Chemistry, 22, 307-309.

Feung, C-S., Hamilton, R.H. and Mumma, R.O. (1977) Metabolism of 2,4-Dichlorophenoxyacetic Acid. 11. Herbicidal Properties of Amino Acid Conjugates, Journal of Agricultural and Food Chemistry, 25, 898-900.

Lee, C.H., Olcffs, P.C. and Szeto, S.Y. (1986) Persistence, Degradation and Movement of Triclopyr and Its Ethylene Glycol Butyl Ether Ester in a Forest Soil, Journal of Agricultural and Food Chemistry, 34, 1075-1079.

Lewer, P. and Owen, W.J. (1987) Uptake and Metabolism of Triclopyr in Soybean Cell Suspension Cultures: A Comparison with 2,4-D, Biochemical Society Transactions, in press.

Mumma, R.O. and Hamilton, R.H. (1978) Xenobiotic Metabolism in Higher Plants: in vitro Tissue and Cell Culture Techniques, in Xenobiotic Metabolism:in vitro Studies, Paulson, G., Frear, S. and Marks, E. (Eds) American Chemical Society Symposium Series, No. 97, Washington, D.C.