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THE CHEMISTRY, METABOLISM AND RESIDUE ANALYSIS OF SYNTHETIC PYRETHROIDS

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THE CHEMISTRY, METABOLISM AND RESIDUE ANALYSIS OF SYNTHETIC PYRETHROIDS

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Abstract - The chemical properties and metabolism of the synthetic pyrethroids, phenothrin, permethrin, cypermethrin, decamethrin and fenvalenate are reviewed. With the exception of decamethrin, which is a single stereoisomer, all the other pyrethroids discussed are used as a mixture of isomers. These chemicals are all relatively non-volatile; they have very low solubility in water, and can all be hydrolysed at the ester linkage. They are all more stable to photolysis than the natural pyrethroids although phenothrin which contains an isobutenyl group is still relatively photolabile. The photostability of the other chemicals is such that they have been successfully developed as agricultural insecticides.

When applied to the leaves of plants, the pyrethroids are not translocated to any significant extent. They are degraded on the plant surface, probably mainly by photodegradation to a wide range of products many of which are formed by ester cleavage and ring hydroxylation. These products are subsequently conjugated by the plant mainly as glycosides. When plants are grown in treated soil, again little translocation of chemical to aerial parts of the plant occurs. That trace of chemical which is translocated is not intact pyrethroid but is metabolites of the acidic half of the original molecule.

In soil, the pyrethroids are readily degraded. The time taken for loss of half of the chemical ranges from about 2-12 weeks depending on the structure of the compound and the soil type. Hydrolysis of the ester linkage is a major degradation route. The products are further modified by loss of the cyanhydrin where applicable and by hydroxylation. The intact pyrethroids can also by hydroxylated. Further degradation also takes place and extensive evolution of $^{14}CO_2$ has been observed from radiolabels incorporated in various positions of the molecules. Most of the degradation observed has been shown to be mediated by soil microorganisms.

When dosed to rodents, the pyrethroids are rapidly metabolised and the metabolites are excreted in similar amounts in both the urine and faeces. A large number of metabolites have been identified. These are mainly composed of intact pyrethroids and hydrolysis products both of which may be hydroxylated in various positions. The metabolites may also be conjugated with sulphate glucuronide, taurine and other chemicals.

In addition radiolabelled permethrin has been dosed to ruminants and hens. Again excretion was rapid and only small amounts of dosed radioactivity transferred to the meat, milk and eggs of the treated animals.

Sensitive residue analysis techniques are available for the analysis of the pyrethroids. In general the methods involve an initial extraction with organic solvent, followed by a liquid-liquid partition and an adsorption chromatography 'clean-up' step. The final determination is by gas-liquid chromatography using an electron capture detector. Various glc columns may be used to give total or partial separation of the isomers or to avoid separation of isomers. In the case of phenothrin, due to its poor sensitivity to an EC detector, analysis procedures are more complex.

No publications are available describing analysis of the breakdown products of the pyrethroids but some of the methods suggested for parent compounds involve a hydrolysis step and these could be adapted for analysis of metabolites.

1 INTRODUCTION

Since the elucidation of the chemical structures of natural pyrethins (Refs. 1,2,3 & 4), extensive efforts have been made in many laboratories in the world to modify the chemical structure to obtain derivatives with improved chemical properties and better biological performance. During the course of these investigations, allethrin, an analogue of pyrethrin-I with a shortened side chain on the cyclopentenolone of the alcohol moiety, was synthesised (Ref. 5), and later tetramethrin which is the chrysanthemic acid ester of 3,4,5,6-tetrahydrophthalimidomethylol (Ref. 6) was developed. These two synthetic pyrethoids have been commercialised world-wide for some time as the active ingredient of household insecticides.

In 1967 Elliott et al synthesised the chrysanthemic acid ester of 5-benzyl-3-furylmethyl alcohol (resmethrin, NRDC 104) (Ref. 7). It was practically devoid of knock-down activity but proved to have remarkably greater killing effects on various insect species, including mosquitoes and houseflies, than the above pyrethroidal compounds.

5-Benzyl-3-furylmethyl alcohol constitutes the alcohol moiety of other synthetic pyrethroids such as the ethanochrysanthemate (K-Othrin, RU-11679) (Ref. 8), and Kadethrin (RU-15525) with a thiolactone ring instead if the isobutenyl group in chrysanthemic acid (Ref. 9). Further attempts to modify the alcohol moiety led to the chrysanthemic acid esters of 5-propargyl-2-furylmethyl alcohol (furamethrin, prothrin) (Ref 10), and of 2-methyl-5-propargyl-3-furymethyl alcohol (proparthrin, kikuthrin) (Ref. 11).

These synthetic pyrethroids each have some characteristic properties such as higher insecticidal activity, excellent knock-down activity, and/or higher volatility desirable for fumigating agents. However they have been used for agricultural pest control only to a very limited extent, due, at least in part, to their easy decomposition by air and sunlight. Indeed the half-life of photolysis of pyrethrin-I, allethrin, tetramethrin and resmethrin is less than 24 hour (Refs. 12 & 13).

The properties of phenothrin or 3-phenoxybenzyl chrysanthemate (S2539) were reported in 1973 by Japanese researchers. Although insecticidally a little inferior to resmethrin, it proved to be more stable to photodecomposition, and was considered to be promising for agricultural application (Ref. 14).

One of the hydrogen atoms of the benzylmethylene group of the alcohol moiety of phenothrin was then replaced by a cyano group to yield S2703 (Ref. 15) with increased insecticidal activity.

3-Phenoxybenzyl alcohol was esterified with 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylic acid by Elliott et al (Ref 16) to obtain permethrin (NRDC143), with better photostability than phenothrin and with more efficacy than resmethrin. Synthesis of the α-cyano analogue or cypermethrin (NRDC149) followed.

One of the stereoisomers of the bromo analogue of cypermethrin, deltamethrin (NRDC161) is at present the most active pyrethroid against insects, being 1,700 times as active as pyrethrin-I against houseflies (Ref. 17). (The chemistry of these pyrethroids will be dealt with in the next section).

α-Cyano-3-phenoxybenzyl alcohol was also utilised for esterification of 2,2,3,3-tetramethyl-cyclopropane carboxylic acid to obtain fenpropathrin (S3206) (Ref. 18).

Ohno et al (Ref. 19) found that the ester of α-cyano-3-phenoxybenzyl alcohol with 2-(4-chlorophenyl)isovaleric acid (fenvalerate, S5602) is as active as the cyclopropane carboxylic acid esters against various insect species, indicating that the cyclopropane ring is not a prerequisite for biological activity.

The structure-biological activity relationships of these recently synthesised pyrethroids have been summarised and reviewed by Elliott (Refs. 20 & 21).

The newly synthesised pyrethroidal compounds, derived from 3-phenoxybenzyl alcohol and α-cyano-3-phenoxybenzyl alcohol, have excellent insecticidal activity against a wide range of pests, and moderate stability in the environment as well as acceptable mammalian toxicity.

They are also effective against insects, resistant to organophosphorus and organochlorine compounds. They have been rapidly developed as agricultural pesticides for the control of various insect pests on vegetables, fruits, grain crops and cotton, and also for controllong insects of importance in public health and veterinary fields.

Because of the rapidly increasing usage of the pyrethroidal compounds, CCPR asked WHO and FAO to jointly evaluate several of these pyrethroids, and, in 1979, permethrin, cypermethrin, fenvalerate, and phenothrin in part, were evaluated from the view-point of terminal residues including residue analytical methods. In 1980, phenothrin and deltamethrin were evaluated, and, in 1981, these pyrethroidal compounds will be re-evaluated (Cf. note b).

The present review by the Pesticide Chemistry Commission of IUPAC, an official advisory body to FAO, is thus highly relevant. In the review, an attempt has been made to evaluate critically all the information on pyrethroids in the open literature published before mid-1980, and in particular to establish which additional investigations should be carried out to understand fully the chemical nature of the terminal residues of pyrethroids.

In the following sections, chemistry including abiotic transformation, metabolism in plants, soils and animals, as well as the residue analytical methods for the selected pyrethroids are dealt with. In the review of metabolism data, in vitro studies are mostly omitted, since the major purpose of the present review is to evaluate the terminal residues, i.e., in vivo studies.

The name and structure of selected pyrethroids discussed in the review is shown in Figure 1 (Cf. notes a and b).

Note a:

There are many synonyms for the pyrethroids, consisting of common names, trade names and code numbers. Those are shown below.

Cypermethrin	=	Cymbush Imperator Kafil-super Ripcord Barricade PP383 NRDC149 SH1467 CCN52 FMC30980 WL43467
Deltamethrin	=	Decis decamethrin supermethrin hypermethrin NRDC156 NRDC158 NRDC160 NRDC161 RU22974 RU22950 FMC45497 FMC45498
Fenpropathrin	=	fenpropanate phenpropanate S3206 FD706 WL41706
Fenvalerate	=	Belmark Pydrin Sumicidin WL43775 SD43775 S5602
Permethrin	=	Ambush Kafil Ectiban Kefil Mabush Ambusz Picket Matadan Pounce Perthrine Coopex Outflank transpermethrin biopermethrin PP557 NRDC143 RU22090 FMC33297 S3151 WL43479
Phenothrin	=	Sumithrin Fenoxythrin S2539.

Note b:

The brief summary of the 1979 WHO/FAO evaluation is published in "FAO Plant Production and Protection Paper, 20" (FAO 1980). In the evaluation, fenvalerate is not included in pyrethroids, but it is apparently designated as a related compound. However, in this review fenvalerate is classified as one of the synthetic pyrethroids for the following reasons.

1 Although fenvalerate is devoid of a cyclopropane ring, the chemical structure as visualised by the molecular model is similar to that of conventional cyclopropane carboxylic acid esters (Ref. 22).

2 The mode of action of fenvalerate against insects and the toxic effects in mammals are very similar to other typical pyrethroids (Refs. 23 & 24). The characteristic features of insecticidal activity of fenvalerate are:

a) It has greater insecticidal activity dermally than orally.
b) It possesses knock-down and paralysing activity.
c) It possesses a negative temperature coeffecient.
d) It does not exhibit systemic or vapor activity.

3 Fenvalerate attacks the peripheral nervous system and the associated organs of insects, in a similar way to other pyrethroids (Ref. 25).

Thus the important criteria for assigning a chemical to the pyrethroid group should be the mode of action rather than the presence or absence of the cyclopropane ring.

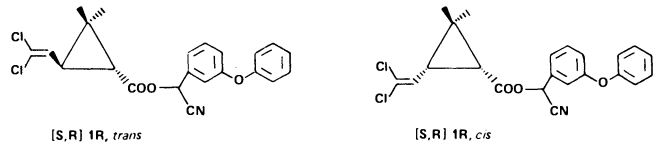
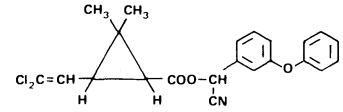
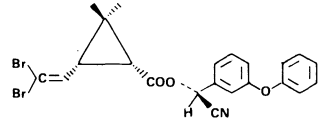
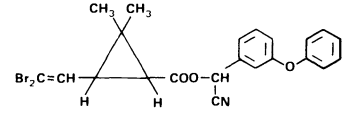
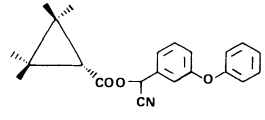
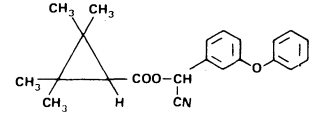
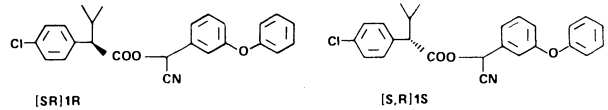
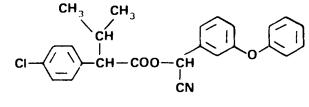
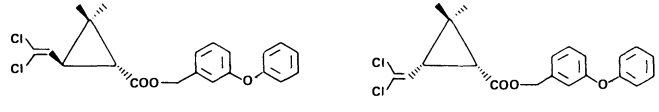
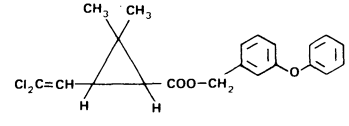
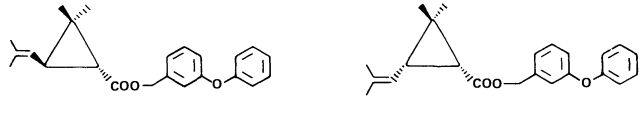
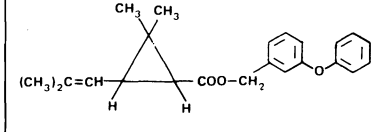
Common name and code numbers	Stereochemical structure	Simple structure	Systematic name
Cypermethrin NR DC 149 WL 43467 (Shell) PP 383 (ICI)	 [S,R] 1R, *trans* [S,R] 1R, *cis*		[S,R] –α–cyano–3–phenoxybenzyl–(1R,1S,*cis,trans*)–2,2–dimethyl–3–(2,2–dichlorovinyl)cyclopropane carboxylate
Deltamethrin NRDC 161	 [S] 1R *cis*		[S] –α–cyano–3–phenoxybenzyl–(1R,*cis*)–2,2–dimethyl–3–(2,2–dibromovinyl)cyclopropane carboxylate
Fenpropathrin S 3206 (Sumitomo) WL 41706 (Shell)			[S,R] –α–cyano–3–phenoxybenzyl–2,2,3,3–tetramethyl cyclopropane carboxylate
Fenvalerate S 5602 (Sumitomo) WL 43775 (Shell)	 [SR]1R [S,R]1S		[S,R] –α–cyano–3–phenoxybenzyl–[S,R] –2–(4–chlorophenyl)–3–methyl butyrate
Permethrin NRDC 143 PP 557 (ICI) WL 43479 (Shell)	 1R, *trans* 1R, *cis*		3–phenoxybenzyl–(1R,1S,*cis trans*)–2,2–dimethyl–3–(2,2–dichlorovinyl) cyclopropane carboxylate
Phenothrin S 2539 (Sumitomo)	 1R,*trans* 1R,*cis*		3–phenoxybenzyl–(1R,1S,*cis,trans*)–2,2–dimethyl–3–(2,2–dimethyl vinyl) cyclopropane carboxylate

Fig 1 Names and structures of pyrethroids discussed in this review

2 CHEMICAL AND PHYSICAL PROPERTIES

2.1 Configuration (The chemical structures of the pyrethroids discussed in this review are shown in Figure 1)

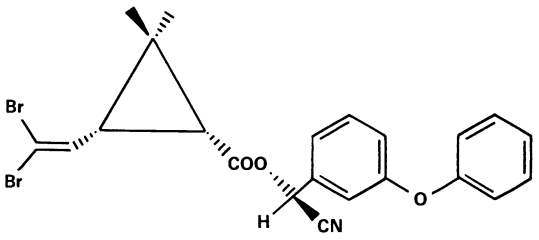
Since chrysanthemic acid has two chiral centres, at C-1 and C-3 of the cyclopropane ring, the chrysanthemic acid esters and their analogs are generally composed of 4 stereoisomers, independent of chirality in the alcohol moiety. Thus, racemic phenothrin (3-phenoxybenzyl chrysanthemate) and permethrin (3-phenoxybenzyl 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropanecarboxylate) contain 4 such isomers namely [1R, trans], [1R, cis], [1S, trans] and [1S cis], although the ratio of trans to cis varies with the method of synthesis (Refs. 26, 27 and 28). These isomers are often referred to as (+)trans, (+)cis, (-)trans and (-)cis isomers, respectively.

1R-Isomers are much more active insecticidally than the 1S-isomers. For the cis and trans isomers, the biological activity apparently depends on the alcohol moiety. trans-Allethrin is more active than cis-allethrin, whereas cis-permethrin and cis-resmethrin are more potent than the respective trans counterparts (Refs. 21 and 26).

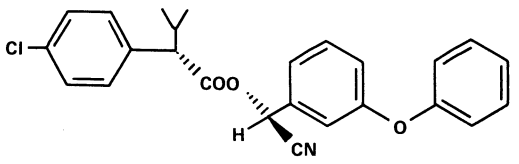
Replacement of one of the hydrogen atoms of the benzyl methylene group of 3-phenoxybenzyl esters with a cyano group leads to the formation of another chiral centre, and again the biological activity is dependent on the chirality. For example, a solution of the ester of (±)-α-cyano-3-phenoxybenzyl alcohol with [1R, cis]-2,2-dimethyl-3-(2,2-dibromovinyl) cyclopropane carboxylic acid (NRDC156) in hexane deposited crystals with the absolute configuration of (S)-α-cyano-3-phenoxybenzyl ester, or NRDC161, which is approximately 6 times as active as the corresponding R-isomers (Refs. 17 and 26).

Similarly, racemic cypermethrin (α-cyano-3-phenoxybenzyl-2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate) contains 8 possible isomers, whereas fenpropathrin (α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl cyclopropane carboxylate) is composed of 2 isomers, since the acid moiety has no chiral centre.

Fenvalerate (α-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)isovalerate) has two chiral centres, at C-2 of the acid moiety and at the αC of the alcohol moiety. Among the 4 isomers, the most active one, designated as Aα, was found to have the configuration of [2S, αS], and the absolute configuration of the [S]-acid proved to coincide with that of [1R, trans]-chrysanthemic acid (Ref. 29).



deltamethrin



Aα – isomer of fenvalerate

Except for deltamethrin, most of the synthetic pyrethroids have been developed as mixtures of more than one isomer.

2.2 Physico-Chemical Properties

Since most of the synthetic pyrethroids dealt with here are actually used as mixtures of more than one isomer, the physico-chemical properties vary somewhat with the manufacturing process. At this moment, no detailed information on the physico-chemical properties are available in the open literature, except a brief description in Farm Chemicals Handbook Section D Pesticide Dictionary (1980).

Generally, these pyrethroids are viscous, yellow-brownish liquid or crystalline at room temperature, and relatively non-volatile, with rather high thermal stability. They are more stable under acidic conditions than under alkaline conditions, moderately soluble in a range of organic solvents, but are practically insoluble in water; most of them have a solubility well below 1mg kg^{-1}.

2.3 Photochemistry

2.3.1 Phenothrin and Permethrin

Although phenothrin is more resistant to photolysis than pyrethrin-I, allethrin and resmethrin, due to more a stable alcohol moiety, it still possesses the fragile isobutenyl group in the molecule, and is easily photodecomposed (Refs. 14 and 30). The dichlorovinyl analog or permethrin is more stable (Refs. 16 and 30). Permethrin, in thin film on plywood, remained insecticidally active after 26 days compared with 4-8 days and less than 2 days for phenothrin and resmethrin, respectively. When exposed as a thin film near a window indoors, phenothrin had a half-life for disappearance of about 6 days, whereas 60% of the applied permethrin remained undecomposed after 20 days.

Thus permethrin was reported to be 10-100 times more stable than previous pyrethroids (Ref. 30). No further photolysis study on phenothrin has been reported in the open literature.

Photolysis of [1RS, trans]- and [1RS, cis]-permethrin was pursued using materials [^{14}C] labelled at carboxyl (acid) or benzylmethylene (alcohol) groups.

Both permethrin isomers decomposed under artificial light (peak outlet 290-320 nm) slightly faster in hexane than in methanol. In each solvent, the cis isomer photo-decomposed ~1.6 times faster ($T_{1/2}$ 43-58 min) than the trans isomer. The reaction involved extensive isomerization of the cyclopropane ring, i.e., interconversion of the trans and cis isomers. This probably occurred via a triplet energy state forming the diradical intermediate (i.e., cleavage of the cyclopropane ring), since the reaction proceeded in the presence of phenoxybenzaldehyde and benzophenone acting as photosensitiser, and was efficiently quenched by 1,3-cyclohexadiene. The isomerisation rate increased in the order methanol<hexane<water, and, at equilibrium after 1-4 hour irradiation, the more thermodynamically stable trans isomer constituted 65-70% of the isomer mixture.

Together with the isomerisation reaction, ester cleavage was the major photo-reaction in methanol, hexane, water and aqueous acetone (2%). Smaller or trace amounts of monochloro-permethrin (from reductive dechlorination), 3-phenoxybenzylaldehyde (PBald), 3-phenoxybenzoic acid (PBacid), 3-phenoxybenzyl 3,3-dimethylacrylate and benzyl alcohol as well as the corresponding aldehyde and acid were also formed.

PBalc was the major product from the alcohol moiety in water and 3-hydroxybenzyl alcohol was the second major product. In addition large amounts if unidentified polar materials were detected especially in water.

Permethrin and monochloro-permethrin did not undergo photo-oxidation or other reactions during 7 days in methanol, in the presence of excess oxygen, using Rose Bengal as a sensitizer. Thus they were resistant to epoxidation at the dichlorovinyl group.

Exposure of the permethrin isomers on Dunkirk Silt Loam soil for 48 days resulted in ~55% loss in sunlight and ~35% loss in the dark. The amount of radioactivity unextractable by 1:1 methanol and ether was ~6% in the dark and ~18% in the light. The unextractable radioactivity appears to be due to microbial activity rather than chemical reactions, but irradiation increased the amount.

On/in the soil relatively little isomerisation at the cyclopropane ring was encountered as compared with the photolysis in solution. There was little difference in the amount of free acid detected in the dark or light and PBalc was the major cleavage product of the alcohol moiety . Other products detected in trace amounts were essentially the same as those in solution. Figure 2 represents the possible photodecomposition pathways for permethrin.

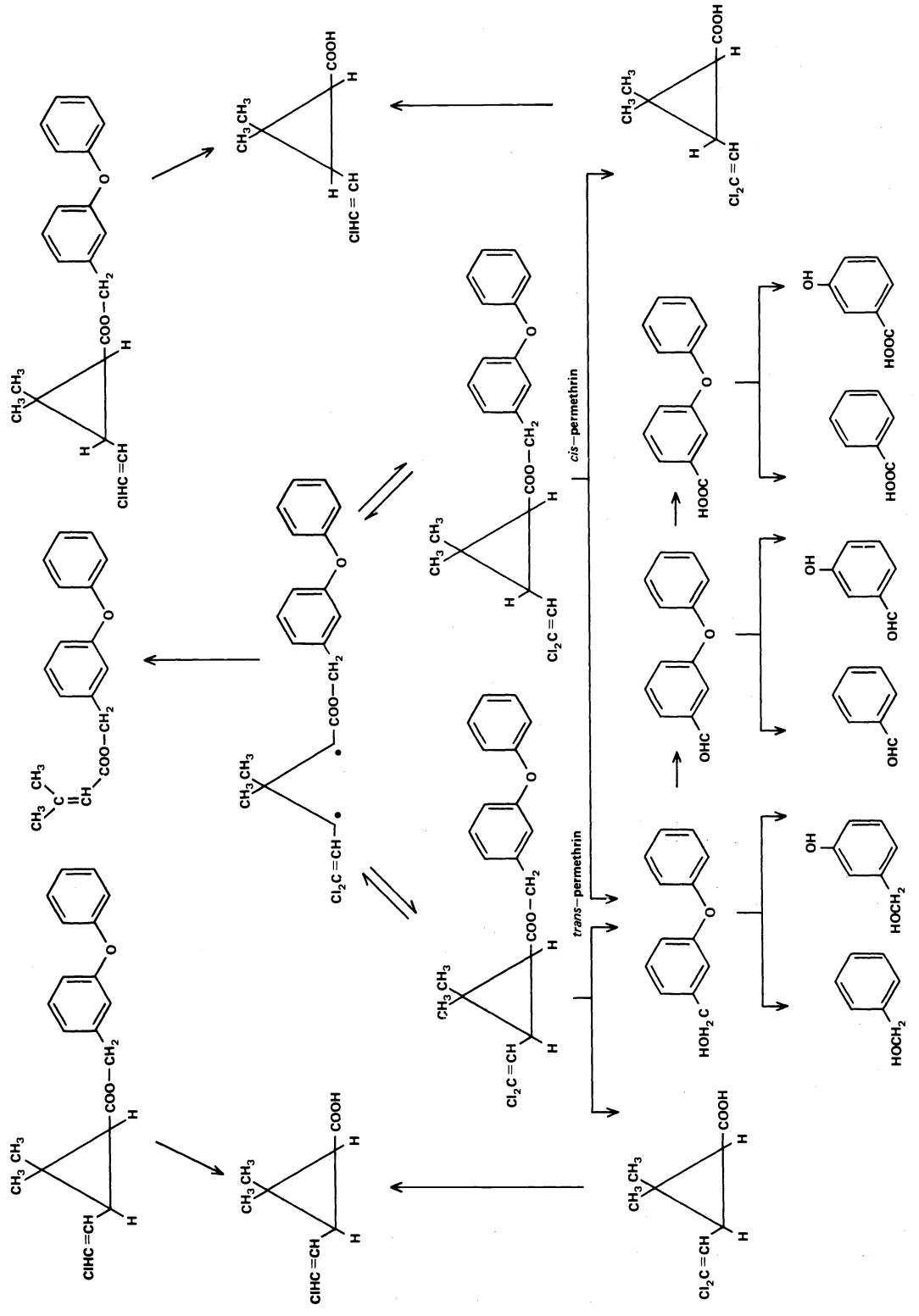


Fig 2 Photochemical degradation pathway of [1 RS] –*trans*–and [1 RS] –*cis*– permethrin to account for photoproducts obtained in UV and sunlight studies

2.3.2 Cypermethrin and Deltamethrin

When radioactive deltamethrin-[1R 3R cis, αS] labelled in the cyano, benzylic or dibromo-substituted carbon was exposed to sunlight as a thin film for 4 to 8 hours (40μg/cm^2) the trans isomers, [1R 3S trans, αS] (compound 3 in Figure 3) and (1S 3R trans, αR] were formed. They accounted for ca. 70% of the applied radioactivity. Smaller amounts of cleavage products including the dibromacid (9), the component cyanhydrin (17) and 18% of unidentified polar products were also formed.

In thick film, small amounts of other products including α-cyano-3-phenoxybenzyl 3,3-dimethylacrylate (7) and 3-phenoxy-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropyl benzyl cyanide [decarboxy deltamethrin] (8) were also detected.

Under uv (λ 290-320nm) the photodegradation rate of deltamethrin apparently decreased with increased solvent viscosity. The relative rate of photodecomposition in hexane and cyclohexane, which have ralative viscosities of 0.33 to 3.0, was 1.5 to 1. The ratedecreased in the series of solvents, methanol, ethanol, 2-propanol as the viscosities increased. There was no difference in the extent of photolysis on flushing with nitrogen or oxygen, while the triplet quenchers piperilene and 1,3-cyclohexadiene reduced the reaction rate. Addition of benzophenone, isobutyrophenone and 3-phenoxybenzaldehyde to the hexane solution yielded a modified mixture.

The trans isomers [1R 3S, αS), [1S 3R, αS] were major photoproducts (30-50%) in acetonitrile-water, whereas they were observed in only minor amounts in methanol, and were absent in hexane.

The major ester products in methanol and hexane were mono-debrominated esters (5 and 6), and, from the acid moiety, the cis acid (9) was always the major product, with smaller amounts of two mono-debrominated acids (10).

The alcohol moiety yielded the component cyanhydrin (17) as well as PBald (18). Other significant photoproducts were PBacid (20) in aqueous acetonitrile, and its methyl ester (22) in methanol, but, in hexane, PBacid was not detected and 3-phenoxybenzoyl cyanide (21) was the major product.

More precise reaction sequences were deduced by irradiation of the dimethylacrylate (7), the cis acid (9), the cyanhydrin (17) or 3-phenoxybenzoyl cyanide (21) (see Figure 3).

The photodecomposition of deltamethrin was compared to that of cypermethrin and permethrin in methanol solution under uv light and in the solid phase (3 mg/cm^2 on glass) under sunlight. On photolysis in methanol, only 5% of deltamethrin remained after 2 days, while 55% cypermethrin and 70% permethrin were recovered. In sunlight, 40% deltamethrin reacted after 30hr, and there was no detectable loss of either cypermethrin or permethrin (Refs. 32 and 33). No other photolysis study on cypermethrin has so far appeared in the open literature.

2.3.3 Fenvalerate

Fenvalerate at 0.01M in methanol, hexane or acetonitrile-water (60:40) underwent rapid photolysis by uv light (λ 290-320 nm) with a half-life of 16-18 min. At 90-95% conversion after 60 min, 2-(3-phenoxyphenyl)-3-(4-chlorophenyl)-4-methylpentanenitrile [decarboxy fenvalerate] was the major product, accounting for 54-70% of the total reaction mixture, with smaller amounts of the dechlorinated analog of decarboxy fenvalerate and the dimer of 2,2-dimethyl-4-chlorostyrene.

3-Phenoxybenzoyl cyanide, PBald, α-isopropyl-4-chlorotoluene and 2,2-dimethyl-4-chlorostyrene were detected in small amounts in hexane and methanol. 3-Phenoxybenzyl cyanide, its dimer and 1,2-bis-phenoxyphenylethane were found only in hexane, and the methyl ester of PBacid only in methanol.

Products unique to acetonitrile-water were 2-(4-chlorophenyl)isovaleric acid [Cl-Vacid] and trace amounts of PBacid and 1-(4-chlorophenyl)isobutanol. Several unknowns were observed with the yields ranging from 5-10% of the total (Ref. 34).

Holmstead and Fullmer investigated photodecarboxylation of several cyanhydrin esters in methanol or hexane under artificial light as models for pyrethroid photodecomposition. The esters gave rise to decarboxylated products to variable degrees, whereas the analogous compounds devoid of cyano group did not produce the photodecarboxylated compound. The photoelimination process apparently occurs via discrete radical intermediates which are formed by the stepwise loss of carbon dioxide, possibly from the triplet state of the starting ester, with subsequent recombination of the radicals within a solvent cage. Thus, α-cyano-benzyl phenylacetate which, yielded the stable benzyl radical, gave substantially

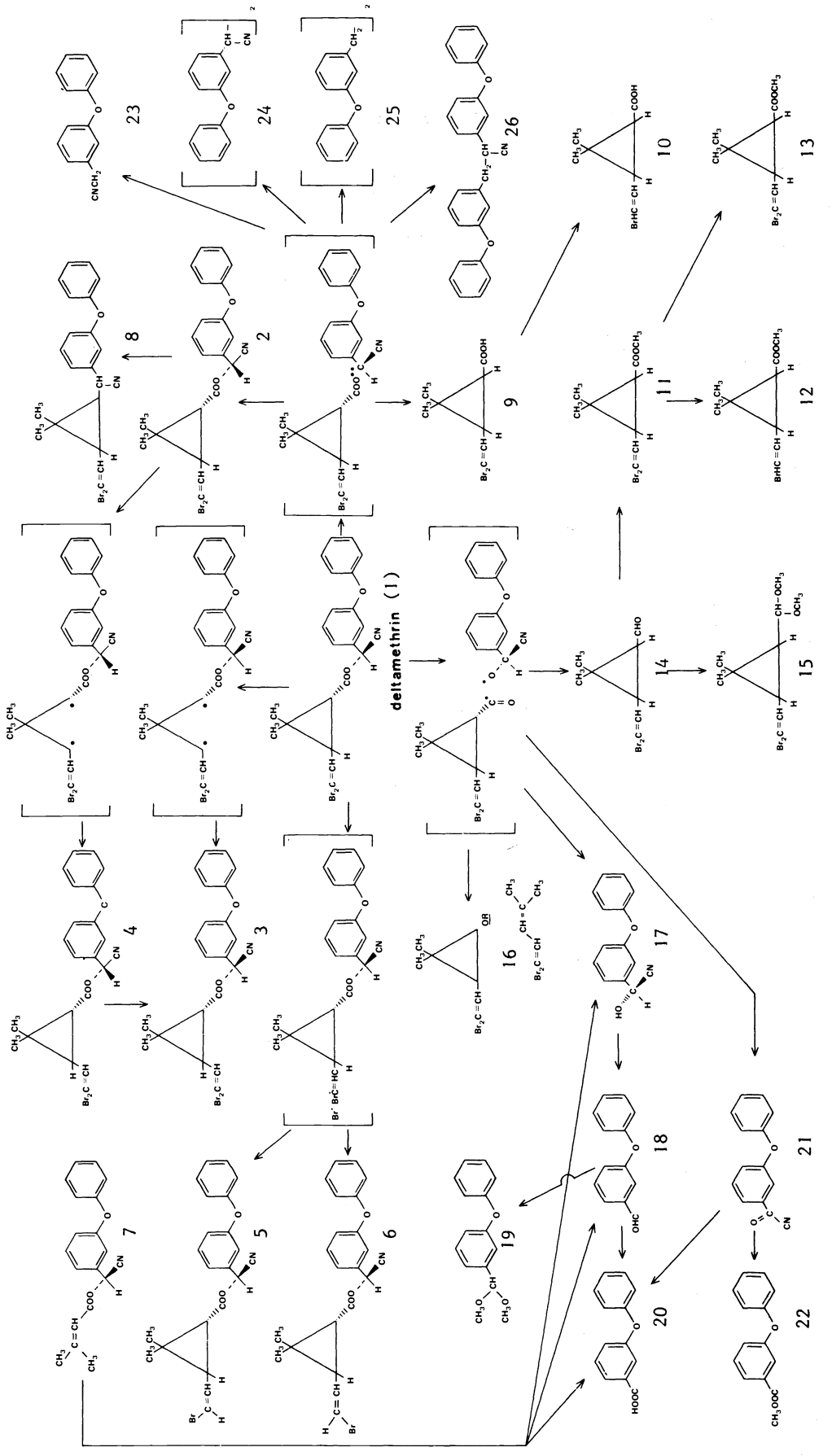


Fig 3 Photochemical degradation pathways for deltamethrin
Possible radical intermediates are drawn in brackets

larger amounts of the decarboxylated product than α-cyanobenzyl benzoate which produced the unstable phenyl radical (Ref. 35).

Fenvalerate as a thin film on glass (1mg/cm^2) was rapidly decomposed by sunlight with a half-life of ca. 2 days. After 43 days, when 10% of the fenvalerate remained, many of the photoproducts found in studies in solution were also detected.

Decarboxy fenvalerate (0.001 M) in acetonitrile was much more stable to uv irradiation than fenvalerate, since the amount of fenvalerate decreased to ca. 10% after 2 hr, while decarboxy fenvalerate disappeared with a half-life of ca. 32hr, yielding mainly the dechlorinated analog.

Fenvalerate deposits at ca. 0.8mg/cotton plant disappeared rapidly, with 50% remaining after 8 days of exposure to sunlight. At the 23-day sampling, decarboxy fenvalerate, PBalc, PBald and PBacid, 3-phenoxybenzyl cyanide and Cl-Vacid were detected, but not quantified (Ref. 34).

Photodecomposition studies of fenvalerate in water and on soil surfaces were carried out using three preparations [^{14}C] labelled at the following positions CO-[2RS, αRS], αC [2S, αRS] and CN-[2S, αRS]. In sterilised sea and river water as well as in distilled water and 2% aqueous acetone, fenvalerate at 50 ppb underwent rapid photolysis by sunlight with half-lives of approximately 4 days in summer and 14-16 days in winter.

The quantum yield of fenvalerate photolysis was determined to be 6.8×10^{-3} at 313 nm in water relative to 3.9×10^{-1} for parathion. Using this figure, the half-life of disappearance was calculated at 40 degrees latitude north, where the photodecomposition study was conducted, and found to be 4.1 days in summer and 12.4 days in winter, which were in good accord with the experimental values.

The breakdown of fenvalerate was accompanied by the production of greater amounts of $^{14}CO_2$ than ^{14}CN. After 4-6 week irradiation ca. 30% (aqueous acetone and river water) to 55-60% (in sea water and distilled water) of [^{14}C]-fenvalerate was recovered as radioactive carbon dioxide, while ca. 5% of the radioactive cyanide was detected in the latter two aqueous solutions, and ca. 30% in aqueous acetone and river water.

In distilled water, one of the major products was decarboxy fenvalerate, which increased to 20% of the applied radiocarbon after one week irradiation in summer, but decreased thereafter. In winter, the amount was approximately 20% of the radiocarbon after 6 weeks.

The other products derived from ester bond cleavage were Cl-Vacid and PBacid which accounted respectively for 57% and 43% of the applied radiocarbon after 6 weeks. In addition, small amounts, each ca. 2% of less, of α-carbamoyl-3-phenoxybenzyl-2-(4-chlorophenyl)isovalerate [$CONH_2$-fenvalerate], α-carboxy-3-phenoxybenzyl-2-(4-chlorophenyl)isovalerate[COOH-fenvalerate], 3-phenoxybenzyl cyanide, 3-phenoxyphenylacetamide [$CONH_2$-PPA], 3-phenoxyphenylacetic acid [PPA] as well as PBalc and PBald were detected.

The photodecomposition rate of fenvalerate at 5.5-5.9μg/100cm^2 of surface of Kodaira Light Clay, Azuchi Sandy Loam and Katano Sandy Loam under sunlight in autumn was variable, with the respective half-lives of 2, 6 and 18 days. After 10 days irradiation, 84-94% of the applied radiocarbon was recovered, among which 10-48% were not extracted with methanol:water (5:1). The amounts of the bound residues apparently increased as the organic matter content increased.

In any soil tested, $CONH_2$-fenvalerate was a major degradation product accounting for 8-26% of the applied radiocarbon after 10-day irradiation. The product was formed in larger amounts in sunlight than in the dark.

Decarboxy fenvalerate was among the minor products which included COOH-fenvalerate, PBalc, PBacid and α-carbamoyl-3-hydroxybenzyl-2-(4-chlorophenyl)isovalerate[$CONH_2$-desphenyl fenvalerate]. Three to 10% of the radioacarbon were not identified.

Figure 4 presents photodegradation pathways for fenvalerate in water and on soil surface by sunlight (Ref. 36). Several of these photoproducts were tested with respect to mammalian toxicity (Ref. 34), but their toxicity to aquatic organisms including fish and crustaceans may be more important in certain circumstances.

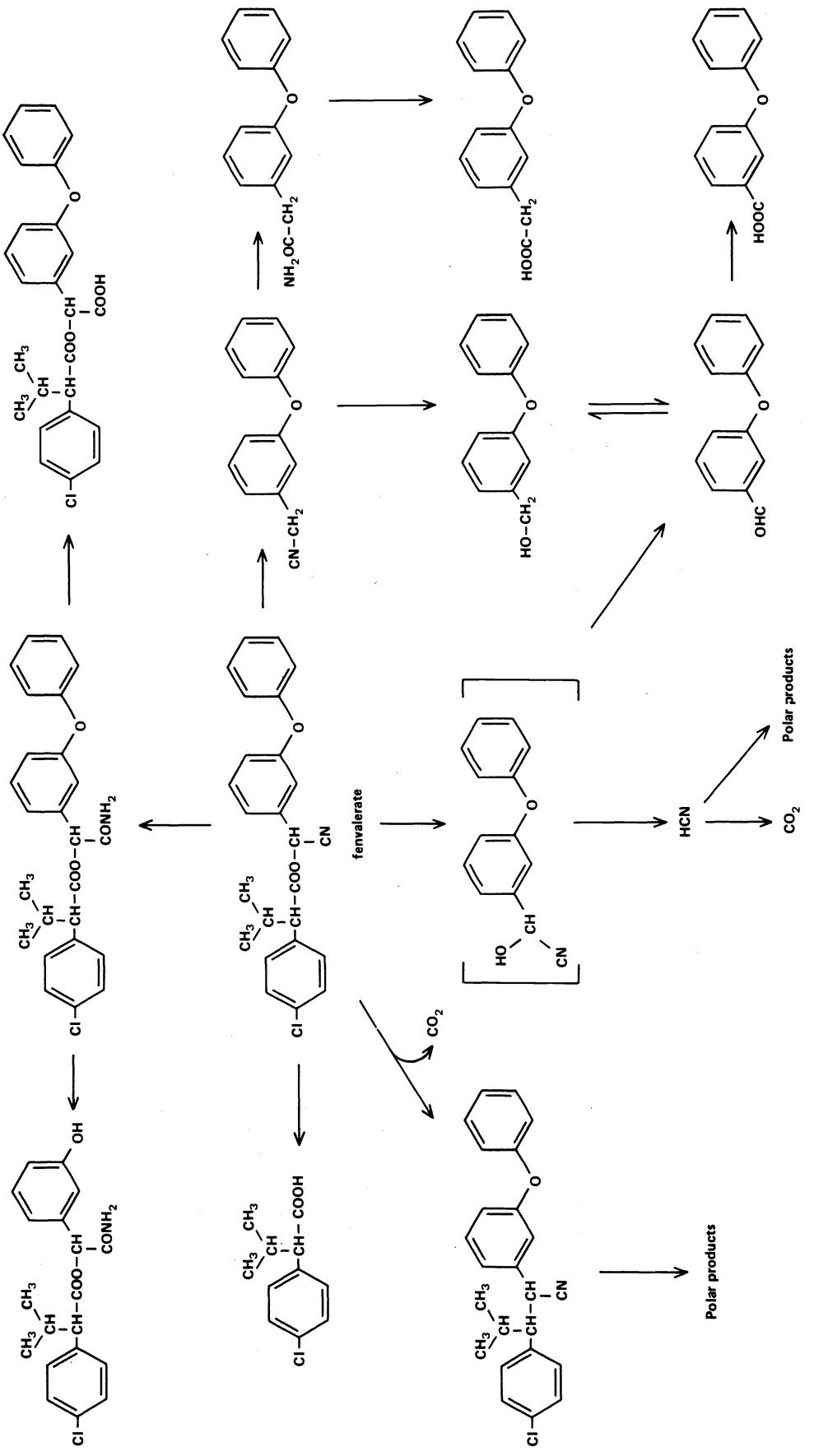


Fig 4 Photochemical degradation pathways for fenvalerate

3 METABOLISM IN PLANTS, SOILS AND BY SOIL MICRO-ORGANISMS

3.1 Metabolism in Plants

3.1.1 Phenothrin

Nambu et al (Ref. 37) have reported that the cis- and trans- isomers of phenothrin have half-lives of less than 1 day on bean and rice plants under glasshouse conditions. This is a considerably more rapid rate of degradation than that of the pyrethroids discussed later.

Both isomers underwent ozonolysis at the isobutenyl bond and the resulting ozonides of the intact phenothrin isomers were rapidly decomposed to the corresponding aldehyde and carboxylic acids (see Figure 5). The ozonides were detected soon after treatment but they were rapidly converted into formyl derivatives and then the carboxy derivatives.

Cleavage of the ester linkage also occurred together with hydroxylation at the 2'- and 4'-positions. Conjugation of the acids and alcohols with sugars were also observed and the formation of polar products was more extensive in rice than bean plants. Limited uptake of labelled products from soil into plants took place when plants were grown in soils treated with [^{14}C]-phenothrin.

The proposed pathways of conversion of phenothrin in and/or on plants by metabolism, photochemical conversion and oxidation are shown in Figure 5.

3.1.2 Permethrin

The short-term metabolism of [^{14}C]-permethrin in snap bean seedlings (Phaseolus vulgaris L) has been studied in the glasshouse (Ref. 38). Cis- and trans-permethrin isomers labelled separately in the dichlorovinyl group and in the methylene carbon atoms (see Figure 6) were used to treat individual bean leaves on 14-day old seedlings. At intervals up to 14 days, five leaves were removed and rinsed with methanol prior to extraction by homogenising with methanol. The leaf washings and extracts were examined separately.

Autoradiography of plants prior to analysis showed that little translocation of radio-labelled permethrin or its metabolites had occurred. The amounts of radioactivity present after 14 days were 13-17% (of the amount applied) in the surface wash, 46-58% in the methanol extract and 8-14% unextracted in the plant residue. Similar total recoveries were obtained with each isomer and each radiolabelled sample.

Some interconversion of the cis- and trans-isomers occurred and the cis-isomer was a little more persistent than the trans-isomer. The initial half-lives were 9 and 7 days respectively. In addition to the parent compound, a large number of metabolites were present in the plant extracts, the most important ones from the alcohol moiety being 3-phenoxybenzyl alcohol (PBalc), 3-phenoxybenzoic acid (PBacid), 2'-hydroxy and 4'-hydroxy derivatives of permethrin (2'-HO-per and 4'-HO-per), and of 3-phenoxybenzyl alcohol (2'-HO-PBalc and 4'-HO-PBalc). A number of polar compounds were also present and these underwent hydrolysis with β-glucosidase to yield PBalc and 2'-HO-PBalc as aglycones. Some 7-8 additional minor products were not identified.

The cis- and trans-cyclopropane carboxylic acids (Cl_2CA) were the major metabolites from the acid moiety and these occurred mainly in conjugated form. From hydrolysis experiments using β-glucosidase, it was inferred that the sugar concerned was glucose but no detailed evidence of the identity was obtained.

In a separate study, Gaughan et al examined the metabolism of permethrin in snap beans in the glasshouse and in cotton both in the glasshouse and outdoors (Ref.s 39 and 40). Four radiolabelled samples were used, namely cis- and trans-permethrin labelled separately in the carboxy or methylene carbon atom.

In the glasshouse, individual leaves of snap beans (Cv. Contender) and cotton (Cv. Stoneville 7A) on young plants were treated topically with 1µg of cis- or trans-permethrin labelled either in the carboxy or methylene carbon (see Figure 6). Similar treatments were made to cotton plants growing under field condition in California. Stem injection of bean plants was used as an alternative application method. However, results from stem injection experiments are less valid with respect to terminal residues than results from topical treatments.

Under outdoor conditions, about 30% of the radiolabel was lost from the cotton plants after one week and the more rapid loss of the trans isomer was confirmed. The major degradation pathway was again hydrolysis followed by rapid conjugation of Cl_2CA isomers and PBalc

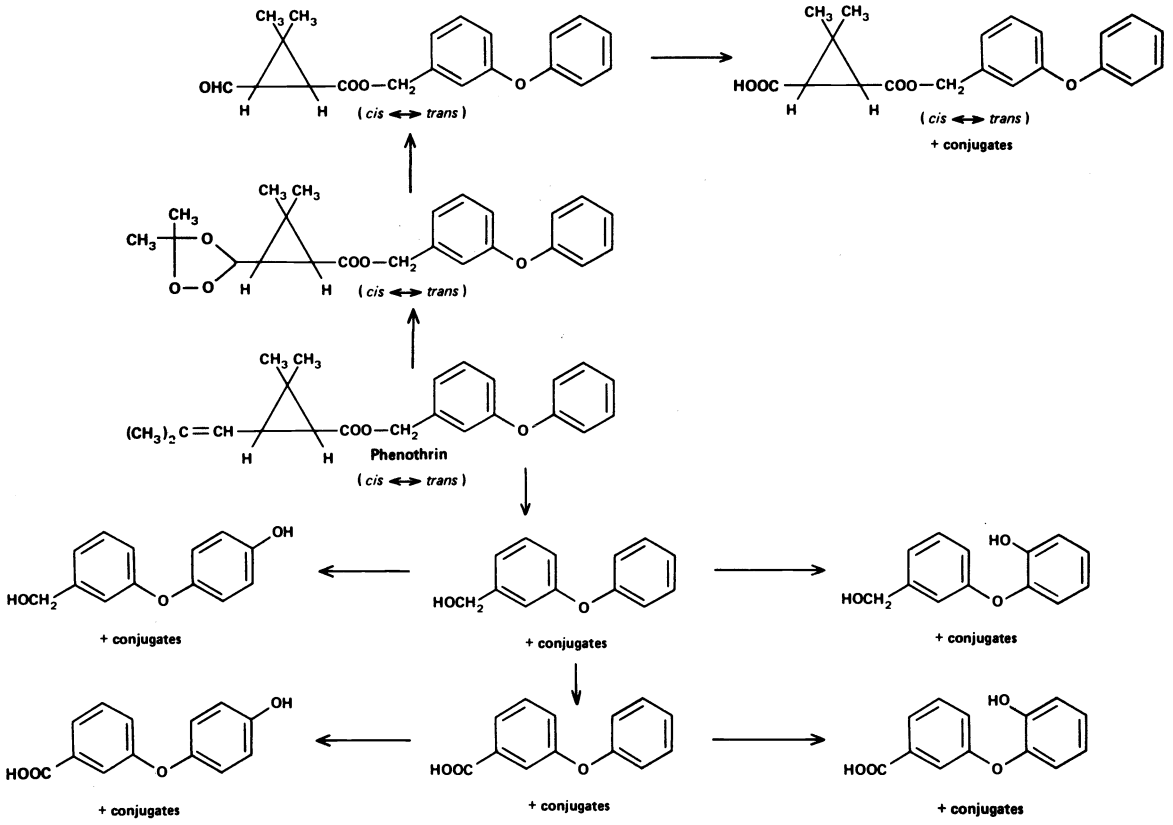


Fig 5 The metabolism of phenothrin in plants

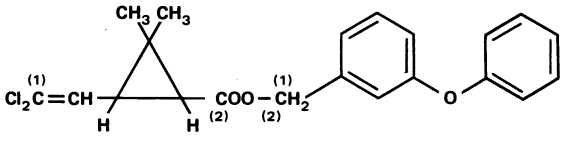


Fig 6 Structure of permethrin showing positions of [^{14}C]–labelling used by (1) Ohkawa *et al* (Ref 38) and (2) Gaughan and Casida (Refs 39 and 40)

formed. Not all the conjugates were readily cleaved by β-glucosidase so it was inferred that these are likely to be compounds other than simple glucosides. However, they are unlikely to be amino acid conjugates since they did not undergo methylation. Another possibility is that glucose esters of carboxylic acids were present but were less readily hydrolyzed by β-glucosidase than the ether-linked glucosides.

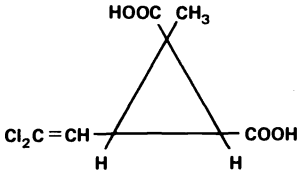
Other products identified included those hydroxylated compounds reported earlier from beans treated with permethrin. In addition, however, oxidation at the methyl groups of the acid moiety had taken place followed by conjugation. This had not been detected in an earlier study with beans.

Some quantitative differences in the formation of conjugated metabolites under glasshouse and field conditions was noted. In particular, some compounds which were detected in minor amounts in the glasshouse were not present in field samples. This emphasises the importance of outdoor experiments.

The authors of this work concluded that the types of products formed from permethrin in plants were similar to those formed in mammals except for the nature of the conjugates. The combined information available on the metabolism of permethrin in plants is shown in Fig. 7.

Experiments designed to examine the possible uptake of permethrin and its degradation products by plants from soil have been carried out by Leahey and Carpenter (Ref. 41). Sugar beet, wheat, lettuce and cotton were grown in soil treated with ^{14}C-permethrin.

Sandy loam soil was treated separately with [^{14}C-cyclopropyl]-permethrin and [^{14}C-phenyl]-permethrin at dose rates equivalent to a spray application of 2 kg/ha. The applied compound was thoroughly mixed with the top 8cm of soil and crops were sown 30, 60 and 120 days after treatment. In mature plants, from seed sown 30 days after soil treatment, small radioactive residues (up to 0.86mg kg^{-1}) were detected and, in general, higher residues occurred in crops grown in soil treated with [^{14}C-cyclopropyl]-permethrin. The 30 day sugar beet sample was chosen for a detailed study of the compounds present and both Cl_2CA and a cyclopropane dicarboxylic acid were shown to be present.



3–(2,2–Dichlorovinyl)–1–methylcyclopropane–1,2–dicarboxylic acid

It is likely that both carboxylic acids were formed in soil and were subsequently taken up by the plants, since considerably lower radioactive residues were present in the parallel experiments with [^{14}C-phenyl]-permethrin.

3.1.3 Cypermethrin

The metabolism of [^{14}C-cyclopropyl]-cypermethrin has been studied in lettuce, grown and treated, under outdoor conditions (Ref. 42). Lettuce (Cv. all the year round) were grown in boxes of soil in an outdoor, wire-covered enclosure. The plants were treated by spraying on two occasions with formulated [^{14}C]-cypermethrin at a dose rate equivalent to 0.3kg/ha. The time interval between applications was 14 days and the plants were harvested 21 days after the last application.

The plants contained mainly unchanged cypermethrin (33% of the total radioactivity present) and polar products (54%). The latter underwent acid hydrolysis mainly to Cl_2CA. Treatment of the polar material with hydrogen chloride in methanol resulted in an 80% yield of the methyl ester of Cl_2CA. It was concluded therefore that at least 80% of this polar material was present as conjugate of the acid.

As an aid to further characterisation of these plant conjugates, separate experiments were carried out on the fate of Cl_2CA itself in plants. Abscised cotton leaves (easier to handle than lettuce) were exposed to solution of the cis- Cl_2CA by stem uptake. This resulted in the formation of several conjugates. The major one was identified as the β-D-glucopyranose ester by a combination of hydrolysis experiments and GC-MS after acetylation

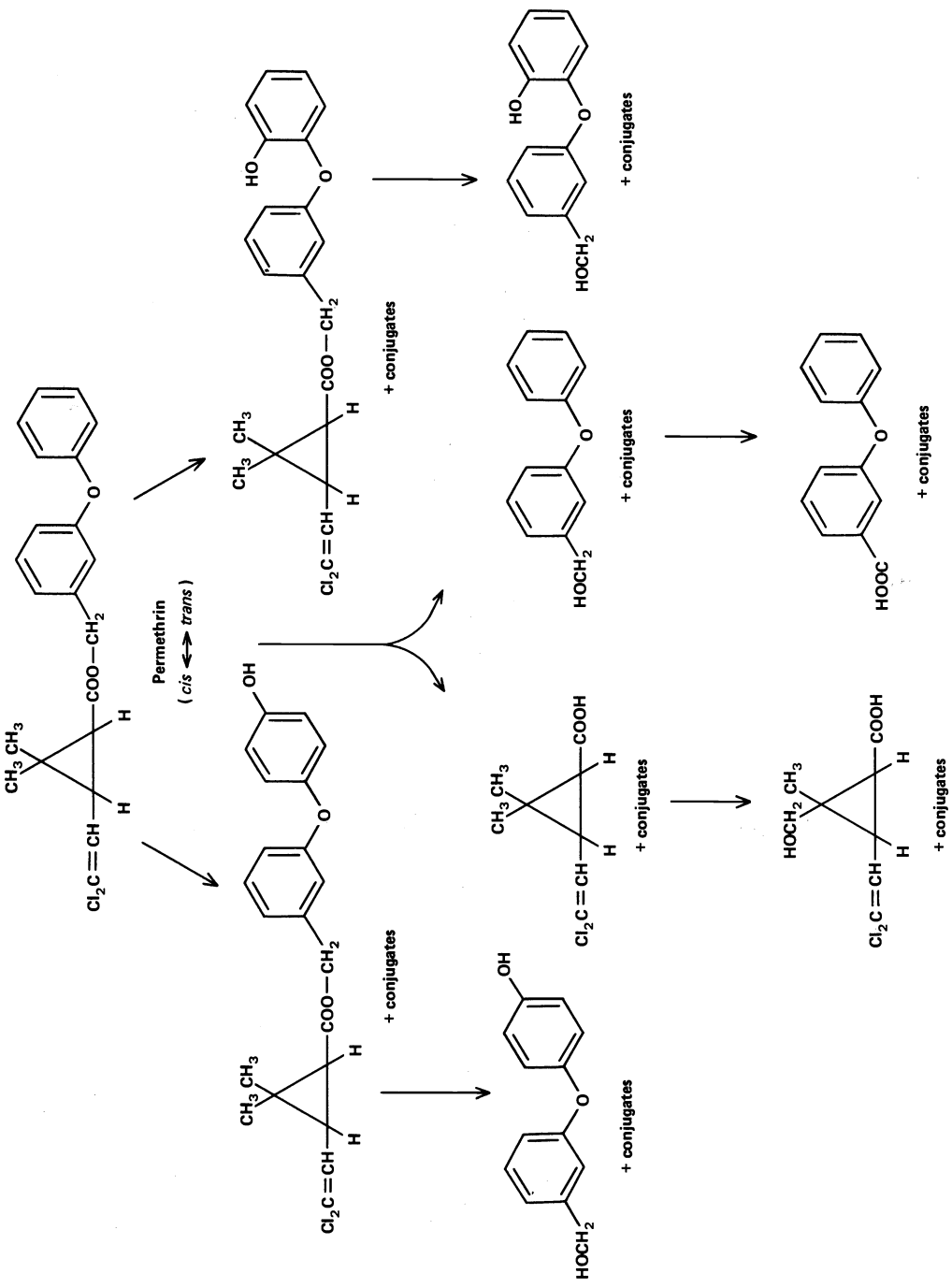


Fig 7 The metabolism of permethrin in plants

in comparison with an authentic acetylated standard. Disaccharide conjugates were also formed. One of these contained a hexose and a pentose and one was shown to contain glucose and arabinose.

Subsequently, the presence of the glucose ester of the cyclopropane carboxylic acid was detected in extracts of plants treated [^{14}C]-cypermethrin. These uptake experiments with metabolites have proved to be a useful aid to characterisation of conjugated metabolites.

In separate experiments, the metabolism of cis- and trans-isomers of cypermethrin in apple fruits and foliage was studied (Ref. 43). Samples of the two isomers labelled separately in the cyclopropyl and benzyl rings were applied to individual leaves and fruits on apple trees growing outdoors. The leaves were treated 3 times and the apples twice and the leaves and apples were harvested 4 and 3 weeks respectively after the final treatment.

Between 36 and 42% of the radioactivity recovered from the leaves was present as the unchanged pyrethroid and up to 30% conversion of the cis- to trans-isomer had occurred. Metabolites detected in the leaves included PBacid, PBald, cis-Cl_2CA, trans-Cl_2CA, the amide analog of cypermethrin, cis- and trans-4'-HO-cyper (see Figure 8 for structure). These free metabolites accounted for between 7 and 15% of the total radioactivity present in leaves. In addition, a mixture of polar compounds were isolated from leaves and these underwent hydrolysis to PBacid, PBalc, PBald, and 4'-HO-PBacid. These compounds were presumably present as conjugates although the reason for formation of PBald as a hydrolysis product is not clear. It is possible that the polar fractions contained small amounts of bound cypermethrin.

The conjugation of [^{14}C]-PBacid itself in cotton, vine and other plant species was studied using abscised leaves in order to obtain more information on the nature of the conjugates produced (Ref. 44). The PBacid was rapidly converted into the glucose ester and disaccharide conjugates, particularly the glucosylarabinose and glucosylxylose esters.

Less extensive metabolism occurred on the apple fruits. More than 98% of the total radiolabel recovered was associated with the peel and up to 77% of this was parent insecticide. Small amounts of the same free compounds detected in leaf extracts were also present in the peel together with polar materials. This information on the metabolism of cypermethrin in plants is summarised in Figure 8.

3.1.4 Deltamethrin

A recent study of the metabolism of deltamethrin in cotton has been reported by Ruzo and Casida (Ref. 45). For this work samples of [^{14}C]-deltamethrin labelled in the dibromovinyl, benzylic and cyano carbons were used to treat cotton (Cv. Stoneville 7A) in the glasshouse and outdoors in Davis, California.

Under glasshouse conditions, the initial half-life of deltamethrin was approximately one week. The overall rate of loss and amounts of extractable and unextractable radioactivity were similar for each radiolabelling position. Conversion of deltamethrin to the trans-isomer occurred and, after 6 weeks, the trans:cis ratio was 1:2.3. Deltamethrin degraded more rapidly under outdoor conditions and, in addition to the more rapid loss of parent compound, there was a higher rate of cis- to trans-conversion. Greater quantities of unextracted products were also present in outdoor samples.

Trace amounts (less than 1% of the applied radioactivity) of hydroxylated derivatives of deltamethrin (4'-HO-delt, t-HO-delt, 4'-HO-t-HO-delt, see Figure 9) were detected with all three radiolabelled samples. However, the major metabolites were free and conjugated dibromovinyl cyclopropane carboxylic acid (Br_2CA), PBalc and PBacid together with smaller quantities of the trans-hydroxymethyl derivative of Br_2CA (t-HO-Br_2CA) and 4'-HO-PBacid. The above compounds are analogues of those from permethrin and cypermethrin in plants.

However, a major difference between the structure of permethrin and the α-cyano pyrethroids is that in the latter case, the first product of hydrolysis is the cyanhydrin (PBcy) rather than PBalc (from permethrin). As with cypermethrin, 3-phenoxybenzylaldehyde (PBald) was also a deltamethrin metabolite, and this was presumably formed from the unstable cyanhydrin.

Several types of conjugated metabolites (based on their behaviour on hydrolysis) were isolated but they were not fully characterised. One type cleaved readily with β-glucosidase or hydrochloric acid to yield Br_2CA and PBacid from the appropriate labelled material. Two other types were resistant to β-glucosidase but were cleaved with hydrochloric acid to yield Br_2CA (from the dibromovinyl label), PBacid, PBalc and PBcy (from the benzyl label) and PBcy (from the cyano label). It may be concluded that either PBcy was present as a conjugate or deltamethrin itself had been bound to a tissue component. Hydrolysis of bound deltamethrin would have given rise to the same hydrolysis products (Br_2CA, PBalc, PBacid, PBcy). The proposed conjugates of PBcy are of interest since they could be similar to the

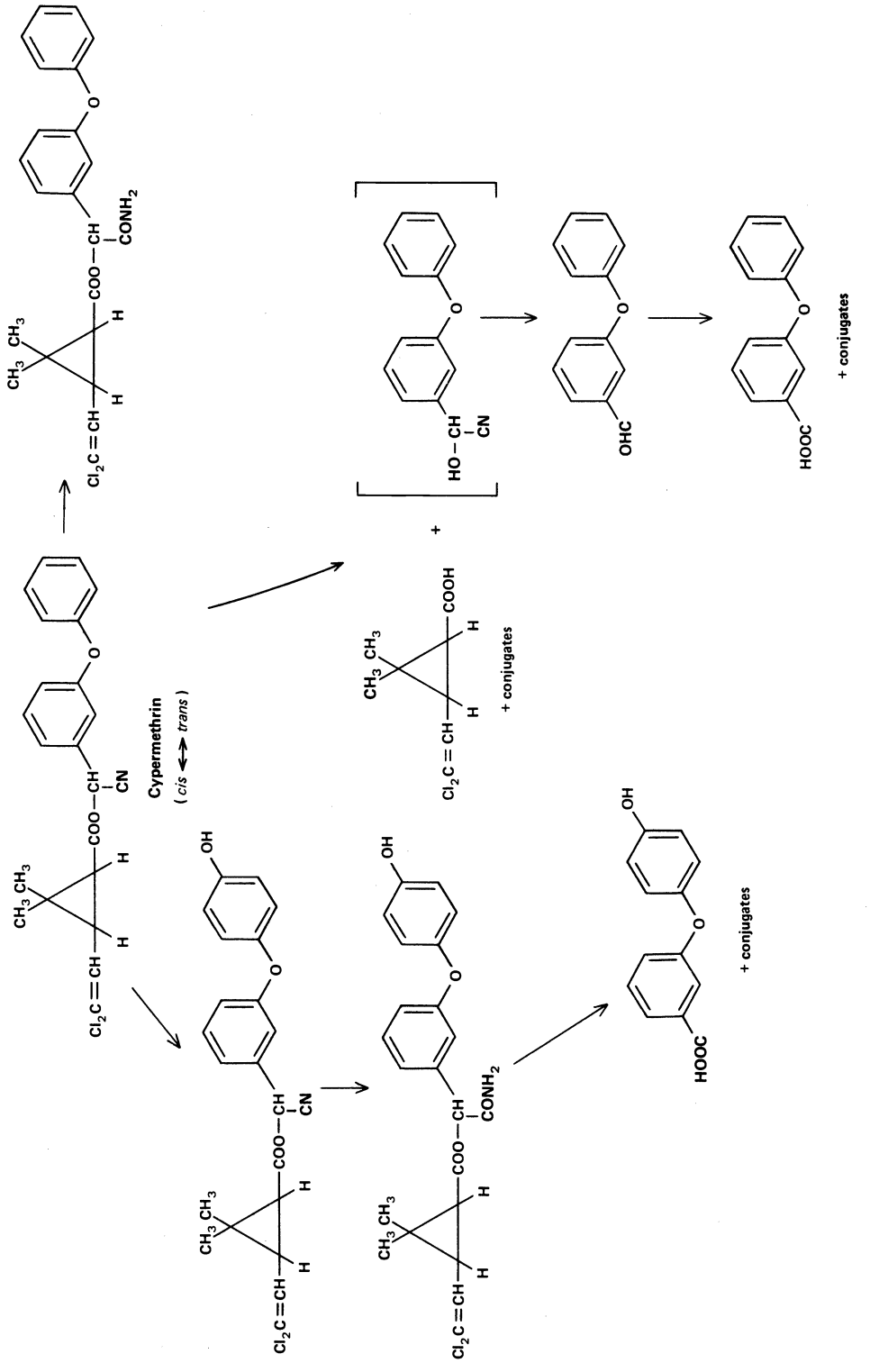


Fig 8 The metabolism of cypermethrin in plants

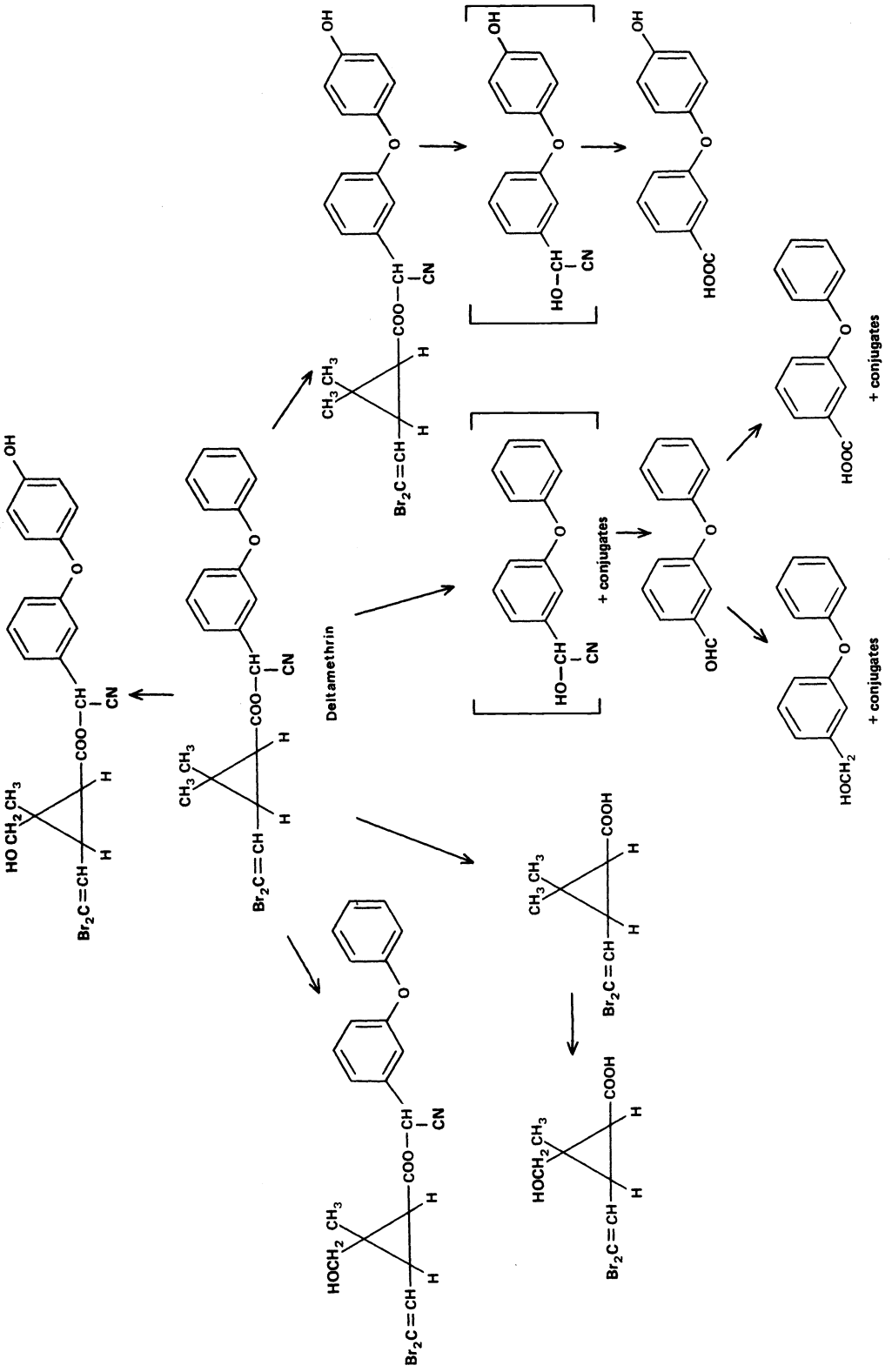


Fig 9 The metabolism of deltamethrin in plants

naturally occurring cyanogenic glucosides, the information on which has been reviewed by Siegler (Ref. 46).

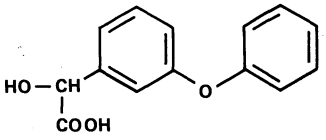
Deltamethrin metabolism has also been studied in cotton and bean leaf discs. Limited conversion (~6%) of deltamethrin into glycoside conjugates (Br_2CA-glyc or PBalc-glyc depending on the labelling position) occurred in the discs. Leaf disc metabolism of both [^{14}C]-Br_2CA and [^{14}C]-PBcy was also studied and more extensive metabolism or conjugation took place. Three types of glycoside were formed from Br_2CA. Both PBalc and PBacid conjugates were formed when PBcy was used as substrates but very little PBcy conjugate was formed. The PBcy was also converted to PBald which could be oxidised to PBacid or reduced to the alcohol. Some differences were also observed between the metabolism in cotton and bean.

The information from these experiments on deltamethrin is summarised in Figure 9. Further work is necessary before the presence of PBcy conjugates can be confirmed, preferably with identification of the intact conjugates.

3.1.5 Fenvalerate

Ohkawa et al (Ref. 47) have studied the metabolism of fenvalerate in bean plants under laboratory conditions. Comparative experiments were carried out using [^{14}C]-fenvalerate labelled in the cyano group and the [S]-acid isomer labelled separately in the cyano, carboxy and benzylic carbon atoms.

Only limited translocation was observed and very low radioactive residues occurred in the seeds. Fenvalerate underwent metabolism or degradation in the plants by several routes. A minor route was conversion of the cyano group to form the amide and carboxylic analogues of fenvalerate. The 3-phenoxybenzyl moiety was metabolised to form similar products to those from cypermethrin and deltamethrin, the major ones being PBalc, PBacid, 2'-HO-PBacid, 4'-OH-PBacid most of which were isolated as conjugates (i.e. polar materials released these compounds upon hydrolysis). In addition PBalc-COOH was formed when polar material was hydrolysed. The presence of PBcy conjugates were inferred since PBald was also a hydrolysis



PBalc—COOH

product of the polar fractions. The major metabolite of the acid moiety was free and conjugated 2-(4-chlorophenyl)isovaleric acid (Cl-Vacid).

The information available on the metabolic pathways of fenvalerate in plants is shown in Figure 10.

The decarboxy derivative of fenvalerate was detected in leaf extracts and this was presumably formed by photochemical conversion on the leaf surface (for structure see Figures 4 and 10). This compound has previously shown to be photochemical product of fenvalerate (Refs. 34 and 36).

When plants were grown in soil treated with [^{14}C]-fenvalerate at a dose rate of 1.0mg kg^{-1}, very little uptake of radioactive residues resulted and residues of <10µg kg^{-1} occurred in the pods and seeds (Ref. 47).

The polar radioactive fractions in plants, mainly composed of sugar conjugates of the acid (Cl-Vacid) and alcohol (actually PBacid) moiety of fenvalerate, were further resolved by TLC and identified by cellulase (Aspergillus niger) hydrolysis as well as methylation and acetylation. The summarised results are shown in Figures 11 and 12. V-M11 (glucosylxylose ester of Cl-Vacid) was the major product in cotton plants but some compound B-M11 (glucosyl-xylose ester of PBacid) was present. In bean plants and cabbage, the malonylglucoside esters of both acids (V-M111a and B-M111a) were mainly formed.

In tomato, not only [1-6]-triglucoside esters (V-M111b and B-M111b) but trisaccharides with varying substitution positions of the glucose moiety (V-M111c and B-M111c) were formed. The synthesised deca-acetyl derivatives of these compounds were not identical to the derivatives of the respective [1-6]-triglucoside esters. The latter saccharides were predominant (Ref. 48).

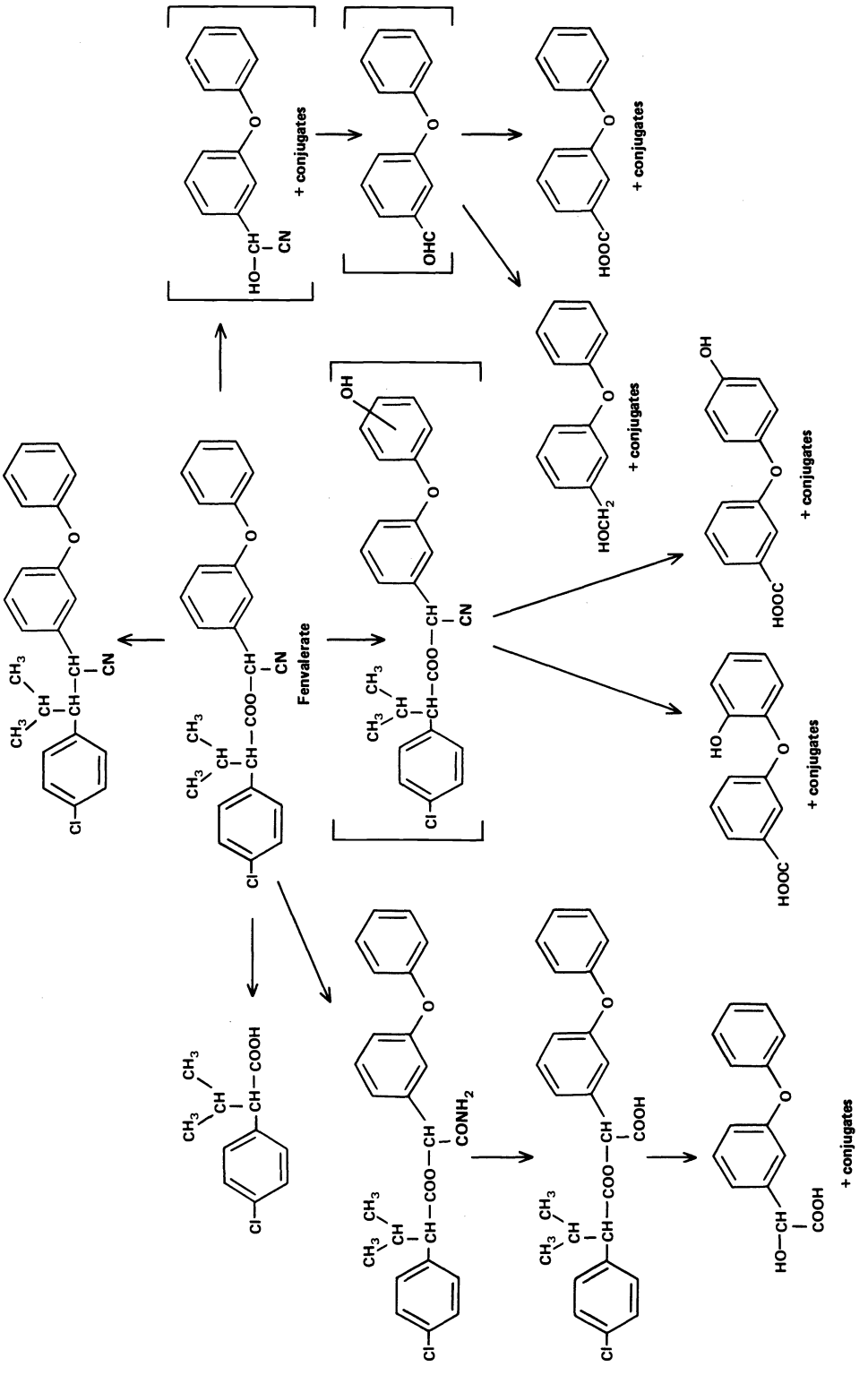
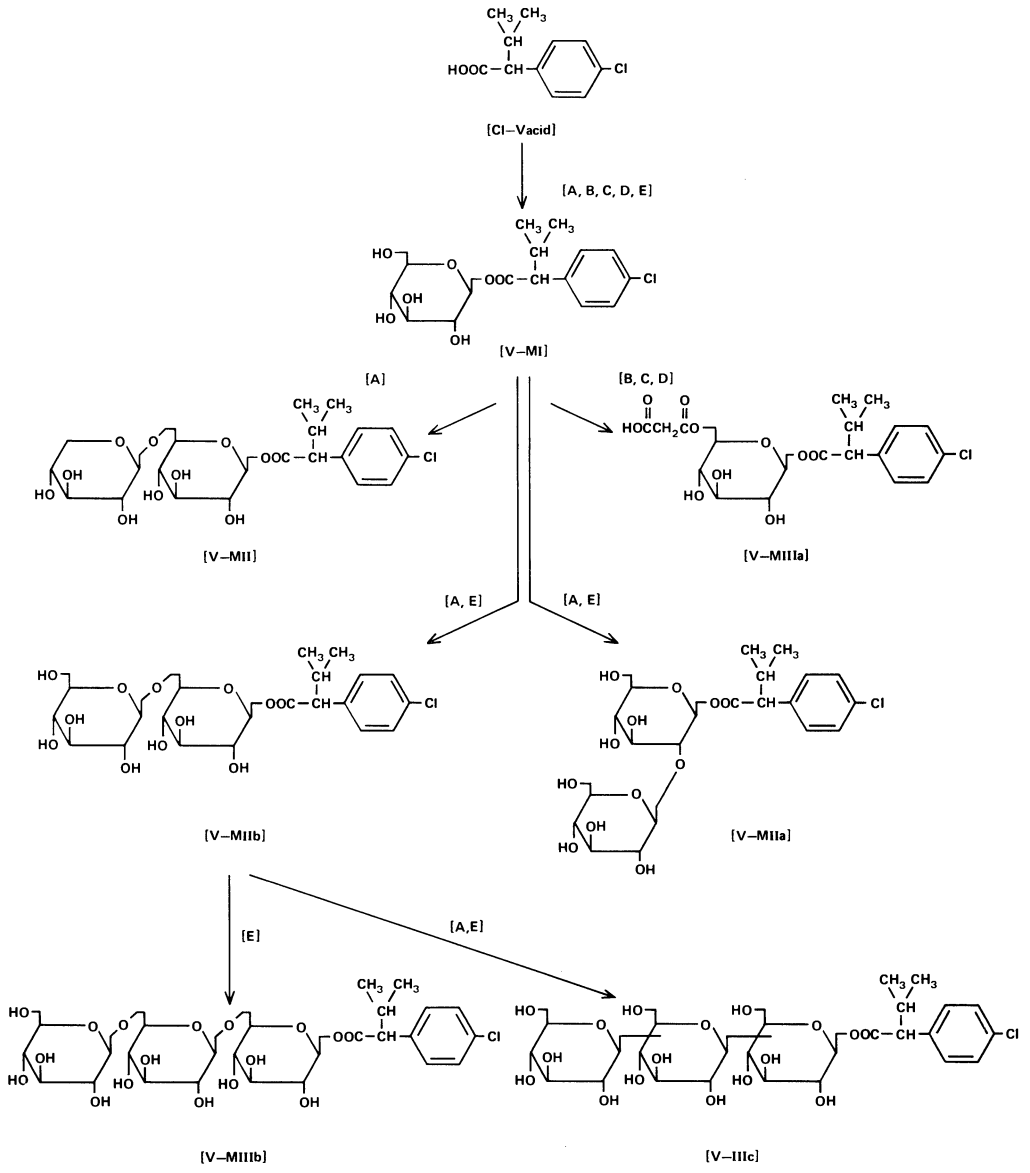
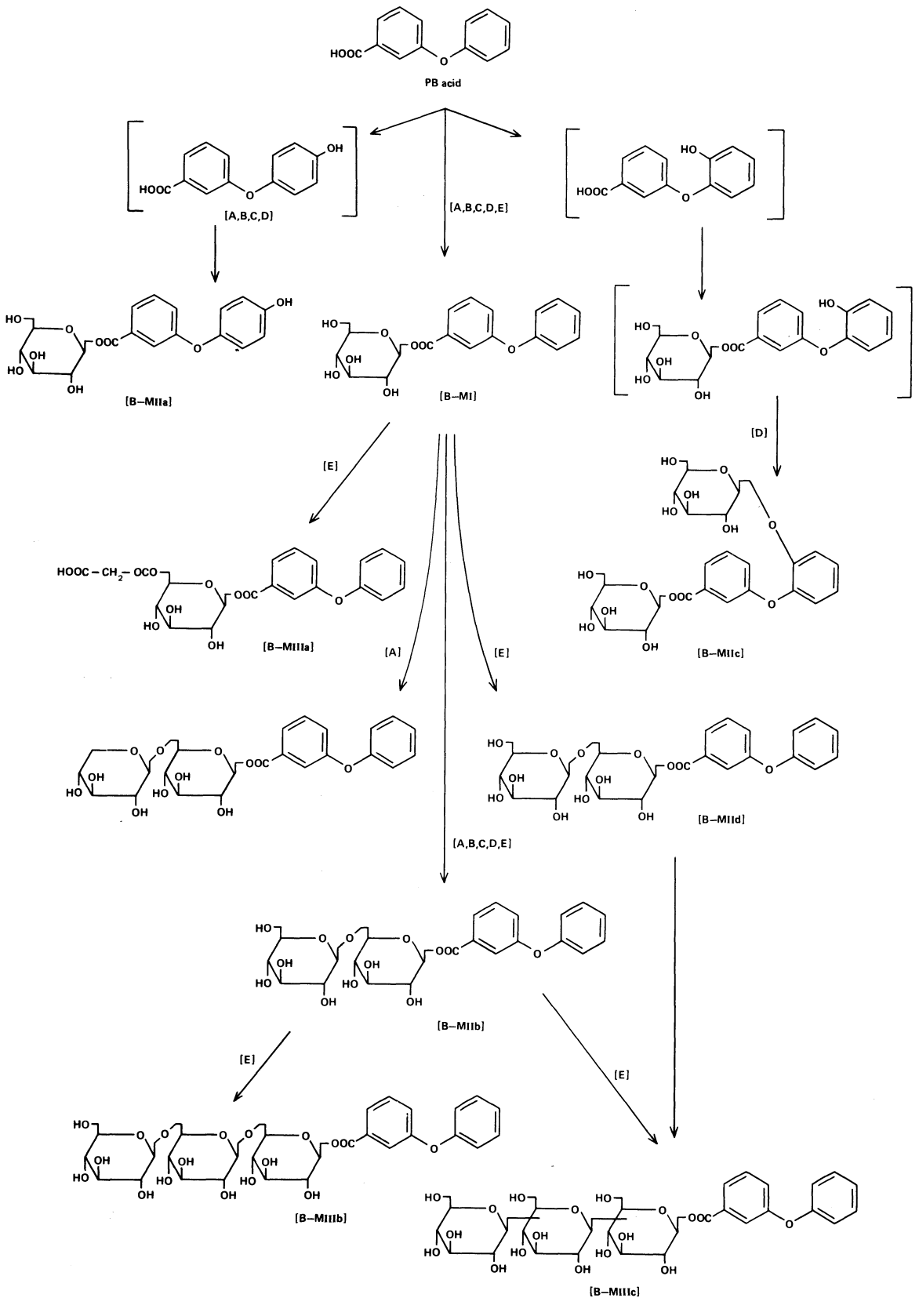


Fig 10 The metabolism of fenvalerate in plants



Cotton [A], Cucumber [B], Cabbage [C], Snap beans [D], Tomato [E]

Fig 11 Proposed metabolic pathways for Cl–Vacid in plants



Cotton [A], Cucumber [B], Cabbage [C], Snap beans [D], Tomato [E]

Fig 12 Proposed metabolic pathways for PB acid in plants

3.2 Metabolism in Soil and by Micro-organisms

3.2.1 Phenothrin

Two Japanese soils, Kodaira light clay soil and Katano sandy loam soil, were treated separately at dose rates of 1mg kg^{-1} with the cis- and trans-isomers of [^{14}C-methylene]-phenothrin (Ref. 37). Both isomers were rapidly degraded in soil with initial half-lives of 1-2 days, but under anaerobic conditions the degradation rate was much slower, with initial half-lives of 2-4 weeks for the trans-isomer and 1-2 months for the cis isomer.

Only 22-47% of the applied radioactivity remained in the soils 6 months after treatment and the major part of this was present as unextractable radioactivity. When experiments were carried out under balance conditions, small amounts of the applied parent compounds (0.1-0.3%) could be trapped following volatilisation, together with large amounts of $^{14}CO_2$. In fact, although parent compounds had short half-lives, there was a steady, almost linear evolution of $^{14}Co_2$ over a 30-day period which must have been due to further degradation of the initial degradation products.

Amongst the degradation products identified during the course of the study were PBalc and PBacid, 4'-hydroxy-derivatives of PBalc, PBacid as well as phenothrin itself. Ether cleavage was also observed to produce the phenolic compounds shown in Figure 13 which contains a proposed degradation pathway of phenothrin in soils.

3.2.2 Permethrin

In several laboratories, the fate of permethrin in soil has been studied and two independent detailed studies have been reported in the literature.

Kaufman et al studied the degradation of separate cis- and trans-isomers of permethrin in 5 soils under aerobic and sterile soil conditions (Ref. 49). Samples of [^{14}C]-permethrin labelled separately in the carboxy and methylene carbons were used.

Soils treated at a rate equivalent to 0.2lb/ac and stored under aerobic conditions at 25°C were analysed at intervals up to 34 days after treatment. Rapid evolution of $^{14}CO_2$ was observed from both radiolabel positions. For example, in a Hagerstown silty clay loam soil 62% and 52% respectively of the methylene-and carboxy-labelled permethrin had been converted into $^{14}CO_2$ in 27 days. Furthermore, the initial rate of evolution of $^{14}CO_2$ was more rapid from the methylene label. The trans-isomer was less stable than the cis-isomer.

A further 15-20% of the applied radiolabel was extractable with methanol and 20-25% remained unextracted and associated with soil organic matter fractions. Consequently, a good radioactivity balance was achieved. There was a minimal volatilisation of unchanged permethrin from the system. Many products were detected in the methanol extracts and the major ones were identified tentatively as PBalc, Cl_2CA and PBacid.

Under waterlogged, anaerobic conditions there was very little (<1% applied radiolabel) evolution of $^{14}CO_2$ and a corresponding increase in the amounts of extractable degradation products.

There was considerable variation in rate of degradation in the 5 soils used and the amount of $^{14}CO_2$ evolved varied from 2.2% in a sandy loam to 51% in a silty clay loam under identical conditions.

These results suggest that fairly rapid hydrolysis of permethrin occurred in most soils under aerobic conditions followed by further degradation of the hydrolysis products with the formation of $^{14}CO_2$ and polar products. Under anaerobic conditions, there was little further degradation after hydrolysis. Since $^{14}CO_2$ evolution did not occur from soil treated with the microbial inhibitor sodium azide, it was concluded that soil microbial activity was involved in the ultimate formation of $^{14}CO_2$ from the insecticide.

In a separate study [^{14}C]-permethrin labelled separately in the dichlorovinyl group and the methylene group was used to study the fate of the compound in two Japanese soils (Ref. 50). Soil samples were treated in the laboratory at a dose rate of 1.0 ppm and samples were analysed at intervals up to 60 days. The initial half-life was 6-9 days for the trans-isomer and 12 days for the cis-isomer. $^{14}CO_2$ was evolved at rates similar to those observed by Kaufman et al. As one of the radiolabel positions (the dichlorovinyl) was different from those used in earlier work, this is evidence for extensive degradation of the cyclopropyl moiety after hydrolysis in the soil.

In addition to the hydrolysis products described above, various hydroxylated compounds were also identified. These included 4'-HO-per (which was present in greater amounts from the

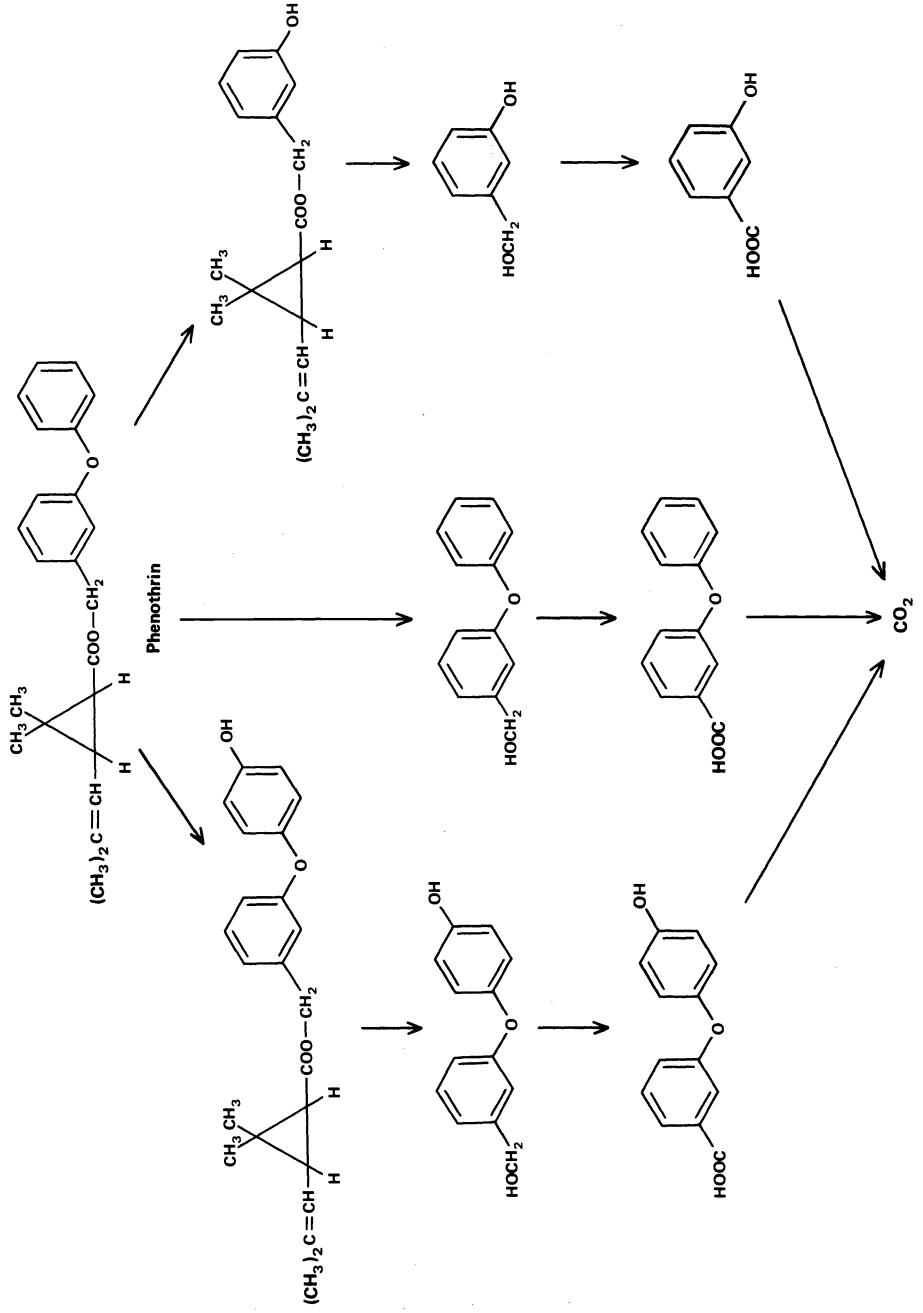


Fig 13 The degradation of phenothrin in soil

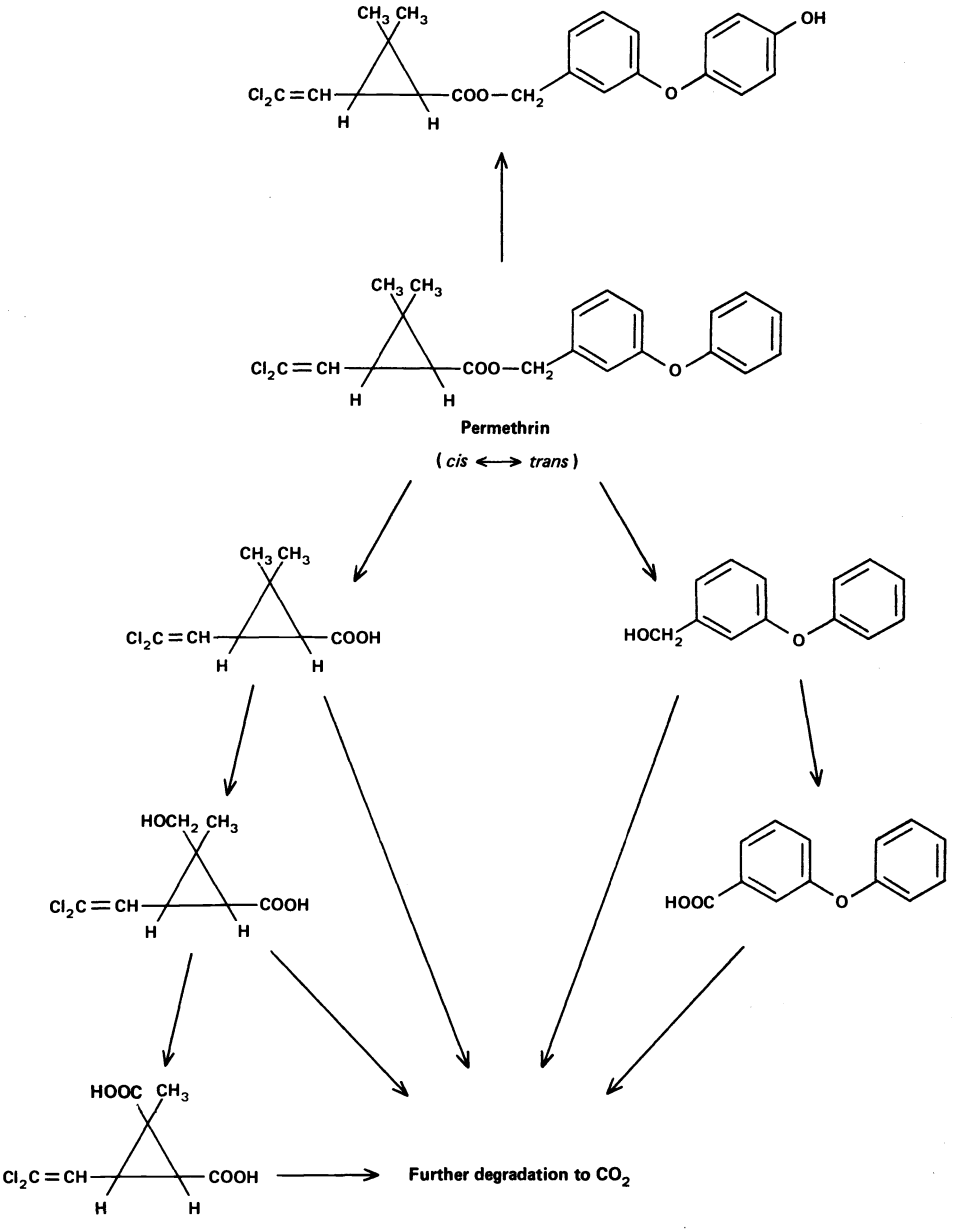


Fig 14 The degradation of permethrin in soil

cis-isomer than the trans-isomer of Cl_2CA and cis-hydroxymethyl-lactones also derived from each acid. The more rapid degradation of trans-permethrin was confirmed and higher concentrations of hydrolysis products were formed from that isomer.

In a laboratory leaching study, there was no leaching of permethrin through soil columns when they were eluted immediately after treatment. If an incubation period of 21 days was allowed before leaching commenced, some movement of radiolabelled degradation products to lower soil layers was observed and traces of PBacid and 4'-HO-per were present in the leachate.

The information available on the degradation of permethrin in soil is summarised in Fig. 14.

3.2.3 Cypermethrin

The degradation of [^{14}C]-cypermethrin has been studied in the laboratory in three soils using separate cis- and trans-isomers labelled in the cyclopropyl ring and aryl ring of the benzyl moiety (Ref. 51). Soils were treated at a dose rate of 2.5 mg kg^{-1} and stored in the laboratory either in glass jars or in biometer flasks. Some soils were stored under anaerobic conditions.

The initial half-life of disappearance of the cis- and trans-isomers of cypermethrin was four and two weeks respectively in sandy clay and sandy loam soils. As with permethrin, the major degradation route was hydrolysis leading to the formation of the Cl_2CA and PBacid. The initial hydrolysis product from the alcohol moiety would be the cyanhydrin (PBcy) which would rapidly degrade and undergo oxidation to form the carboxylic acid.

Under aerobic conditions, further extensive degradation of the hydrolysis products occurred with the formation of $^{14}CO_2$. Bearing in mind that the positions of radiolabelling were in the cyclopropyl- and benzyl-rings (unlike the situation with permethrin studies), this $^{14}CO_2$ evolution is evidence for ring opening followed by degradation of the products formed. A minor additional degradation route of cypermethrin was oxidation to yield 4'-HO-cyper and 4'-HO-3-PBacid.

When [^{14}C-benzyl]-cypermethrin (cis/trans mixture) was incubated with waterlogged soil under anaerobic conditions, hydrolysis occurred at a slightly slower rate than under aerobic conditions, but there was a build-up of PBacid and little further metabolism. Moreover, less unextracted or "bound" radioactivity remained in soils stored under anaerobic conditions.

As the time intervals after treatment increased, there were increases in the amounts of unextracted radioactivity. As this increase paralleled the release of $^{14}CO_2$, it is likely to be the result of incorporation of degradation products (following ring opening) into soil organic matter fractions. Evidence for this was obtained by isolating organic matter fractions from soils containing "bound" residues from which it was shown that fulvic acid, humic acid and humin fractions all contained radioactivity. In the fulvic acid fraction, small amounts of PBacid, 4'-HO-3-PBacid and Cl_2CA were detected.

In separate experiments, soils containing "bound" residues arising from cypermethrin applications were mixed with fresh soil and the evolution of $^{14}CO_2$ was monitored (Ref. 52). Somewhat surprisingly, $^{14}CO_2$ was released at a slow but steady rate, between 21 and 37% of the radioactivity present as "bound" residues being mineralised over an 18 week period. This suggests that the "bound" residues were degrading further rather than accumulating and may be the result of incorporation of ^{14}C into the soil organic matter.

In subsequent work, the cyclopropane dicarboxylic acid (Cl_2diCA) was also identified. Furthermore, it was shown (Ref. 52) that when Cl_2CA itself was added to soil, it was converted initially into the hydroxymethyl analogue and then into Cl_2diCA.

A summary of the degradation of cypermethrin in soil is given in Figure 15.

3.2.4 Fenpropathrin

The degradation of fenpropathrin in soil has been studied using the same soils and conditions as those used for cypermethrin (Ref. 53). The fenopropathrin was more stable in soil that cypermethrin under similar conditions, with an initial half-life ranging from 4 to >16 weeks depending on the soil type.

Once again, hydrolytic cleavage predominated followed by ring opening and $^{14}CO_2$ evolution. However, hydrolysis at the cyano group was also observed and the resulting amide was converted into the corresponding carboxylic acid. No oxidation was observed in the phenyl ring.

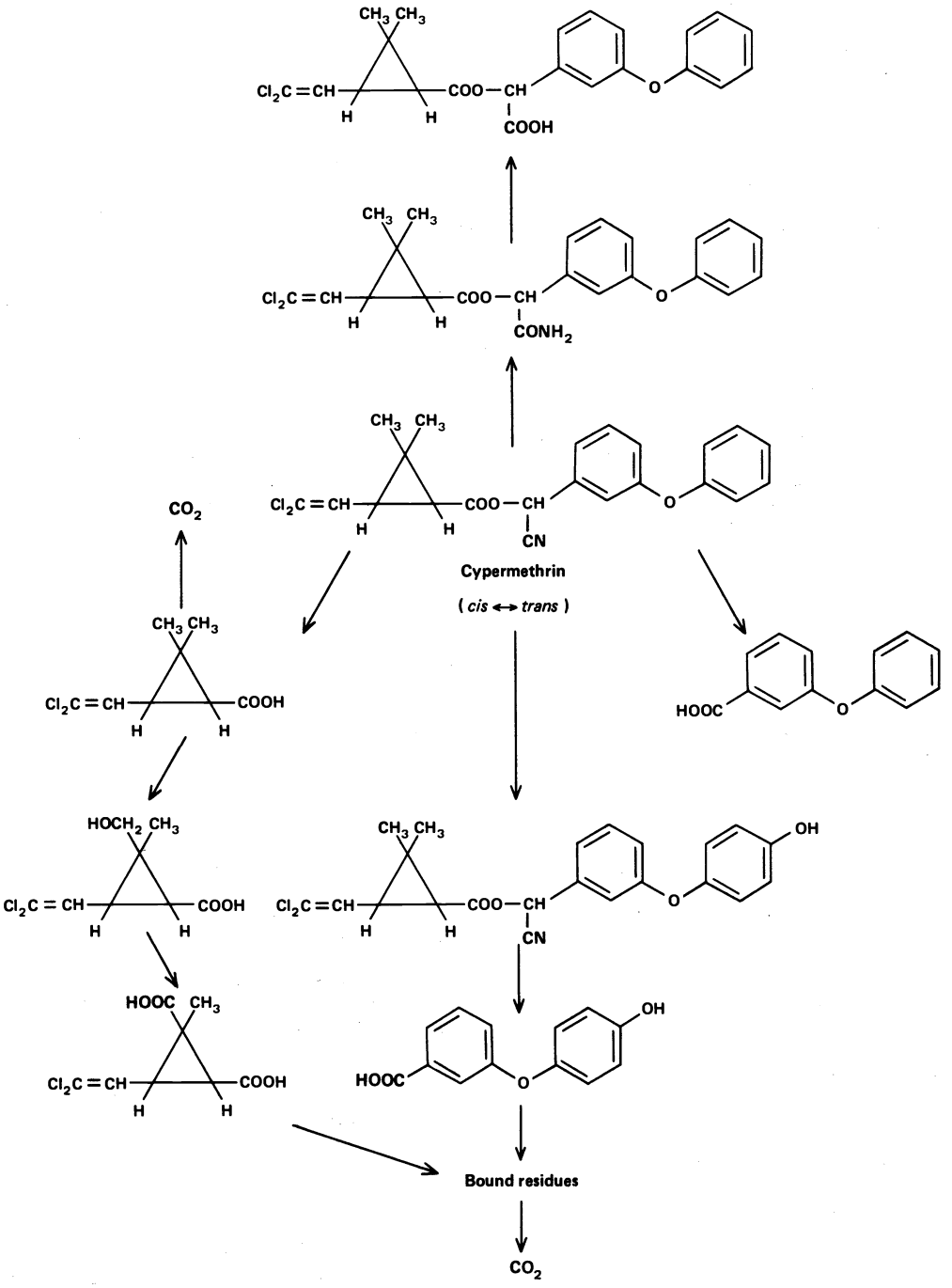


Fig 15 The degradation of cypermethrin in soil

The rate of hydrolysis of fenpropathrin was slower under waterlogged, anaerobic conditions and 2,2,3,3-tetramethyl-cyclopropane carboxylic acid and PBacid were found to accumulate under these conditions.

These results are summarised in Figure 16.

3.2.5 Fenvalerate

Samples of fenvalerate labelled separately with [^{14}C] in the carboxyl and cyano groups have been used to study its degradation in several soils (Ref. 54). Each soil was treated at a dose rate 1.0 mg kg^{-1} and stored at 25°C in the dark under aerobic conditions. The initial half-life for disappearance ranged from 2-12 weeks depending on the soil type.

The degradation products formed were analogous to those from the other pyrethroids and resulted from ester cleavage, ring hydroxylation in the 3-phenoxybenzyl moiety and hydrolysis at the cyano group.

Thus, Cl-Vacid, 4-HO-fenvalerate and the amide analogue of fenvalerate were detected in extracts of all soils. Extensive $^{14}CO_2$ evolution occurred and the amounts were always greater when [^{14}C-cyano]-fenvalerate was used. For example, 30 days after incubation of [^{14}C-]-fenvalerate in Katano sandy loam, 47.5% and 37.9% of the applied radiolabel had been released as $^{14}CO_2$.

In addition, another degradation route not reported in studies with other pyrethroids was ether cleavage resulting in the formation of des-phenyl fenvalerate (See Figure 17) which still has the ester intact. The amount of this compound present ranged 0.2-5.5% of the applied radiolabel in the four soils after 30 days. Ether cleavage of the amide analogue of fenvalerate was also observed.

In common with other pyrethroids, the degradation rate of fenvalerate was much slower under anaerobic conditions. No $^{14}CO_2$ was produced from [^{14}C-carbonyl]-fenvalerate under anaerobic conditions, but approximately 10% of the applied [^{14}C-cyano]-fenvalerate was released as $^{14}CO_2$ under similar conditions.

In a laboratory soil leaching experiment, less than 1% of the applied fenvalerate appeared in the effluent of a soil column when leaching was started immediately after treatment of the soil. Even after a 30 day incubation of the treated soil, only traces of radiolabelled Cl-Vacid eluted through the column when [^{14}C-carbonyl]-fenvalerate was used.

In separate experiments, Japanese soils were used as sources of micro-organisms. Separate culture media were used for fungi and bacteria. Fenvalerate was degraded more rapidly in the bacterial medium than in the fungal culture. Large amounts (35-42% in 2 weeks) of $^{14}CO_2$ were produced from both media when cyano-labelled fenvalerate was added, compared with only 1.1-2.3% of $^{14}CO_2$ from the carbonyl label under the same conditions. In the latter case, Cl-Vacid was a major product, accounting for as much as 69% of the applied radiolabel. Essentially the same products were formed as in the soil studies. However, the carboxy analogue of fenvalerate (by conversion of the cyano group) was present in microbial solutions but not in soil extracts.

A summary of information available on fenvalerate degradation in soils and microbial culture is given in Figure 17.

4 METABOLISM IN ANIMALS

Mammalian metabolism of synthetic pyrethroids has been reviewed by Miyamoto (Ref. 55), and recently by Hutson (Ref. 56). The former author dealt with allethrin, resmethrin, tetramethrin, furamethrin, proparthrin and phenothrin, most of which have been used only for household insect control, while the latter author includes also pyrethroids derived from 3-phenoxy-benzyl and α-cyano-3-phenoxybenzyl alcohol, which have been rapidly developed for agricultural pest control. In this section, the *in vivo* metabolism of these newly developed synthetic pyrethroids, namely phenothrin, permethrin, cypermethrin, deltamethrin, fenpropathrin and fenvalerate will be specifically dealt with. Knowledge of *in vivo* metabolism is particularly important for elucidating the toxicological profiles of the residues of the compounds resulting from agricultural applications. Casida *et al* (Ref. 57) summarised the metabolism of these pyrethroids at the IVth International Congress of Pesticide Chemistry held in Zurich, Switzerland in 1978. His article will be referred to where pertinent in the following discussion.

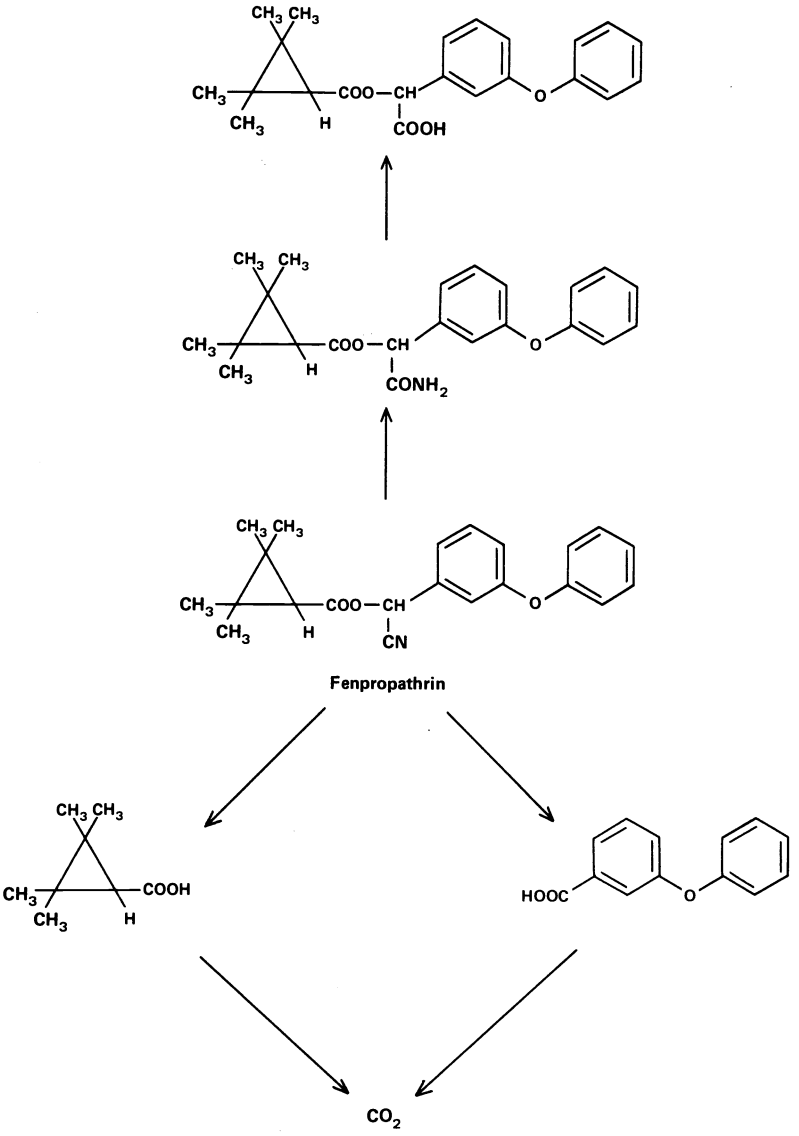


Fig 16 The degradation of fenpropathrin in soil

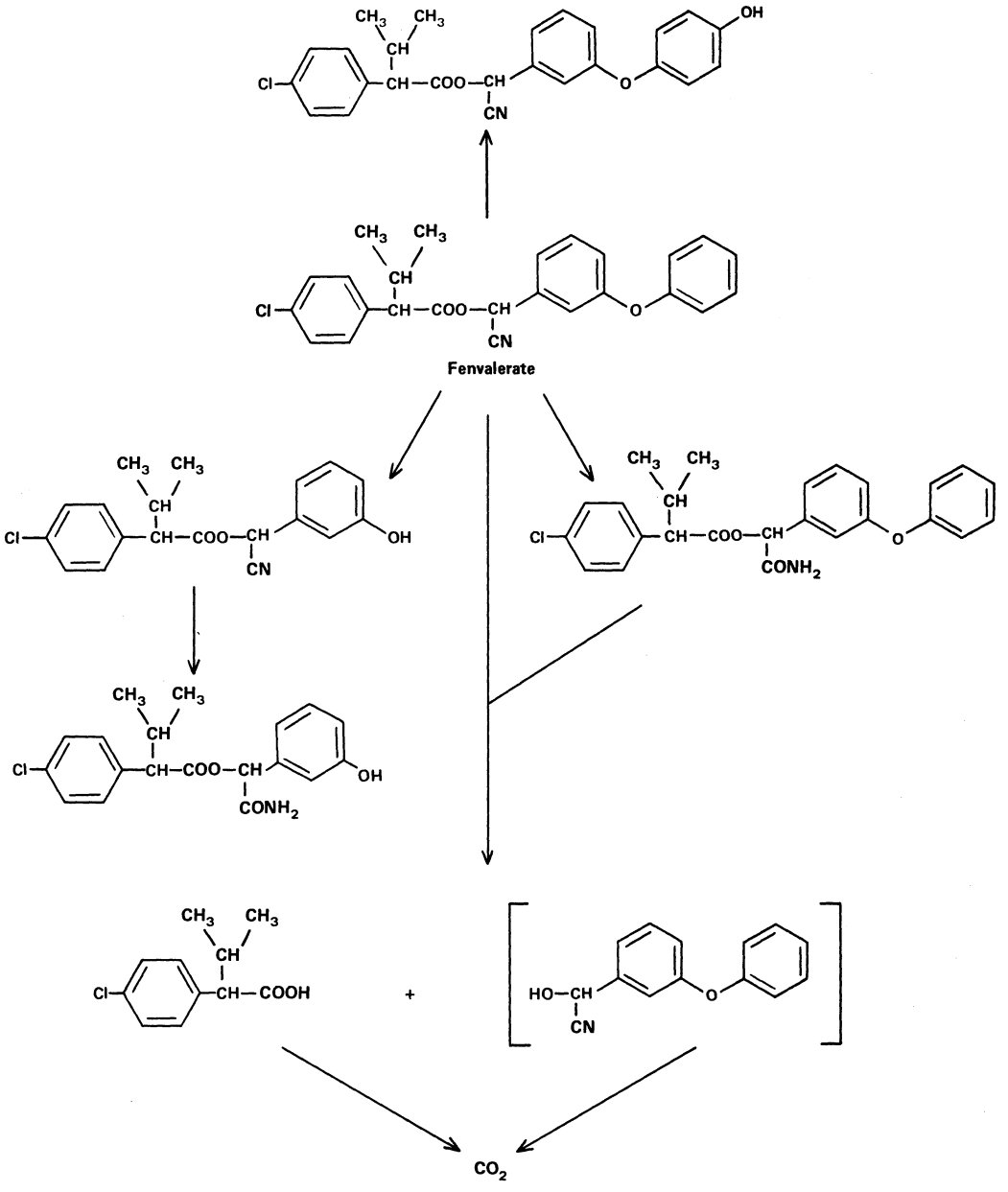


Fig 17 The degradation of fenvalerate in soil

4.1 Phenothrin

When [1R, trans]-phenothrin labelled with [^{14}C] at the benzylic carbon was orally administered to male rats, excretion of the radioactivity was very rapid. Quantitative recovery of radioactivity was obtained within 48 hr (57%) in the urine, (44%) in the faeces, and none was eliminated via the lung. In brain, liver, kidney and blood, analysed during 24 hr after treatment, small amounts of unchanged phenothrin were detected, together with 3-phenoxybenzyl alcohol (PBalc) (Ref 59). The major metabolite was 3-phenoxybenzoic acid (PBacid) accounting especially in liver for more than 50% of the radiocarbon. Intact phenothrin (9% of the dose) was found in the faeces but no attempt was made to look for other ester metabolites. Major radioactive degradation products in the excreta were 3-(4'-hydroxy-phenoxy)benzoic acid (4'-OH-PBacid) (54%), with smaller amounts of PBacid (9.5%) and its glycine conjugate (2.7%) (Ref. 58).

Male rats were dosed with 200 mg kg^{-1} of [^{14}C]-[1R, cis]-phenothrin. From the three days pooled faeces, which contained 65% of the administered radiocarbon, three ester metabolites which accounted for 14% of the dosed radioactivity were identified. These were 4'-hydroxy-phenothrin (4'-OH-c-phe in Figure 18), a phenothrin derivative with the trans methyl of the isobutenyl group oxidised to carboxyl (ωt-acid-c-phe) and a third compound where one of the geminal dimethyl groups was oxidised as well as both of the above modifications (4'-OH,ωt-acid,2-OH(t)-c-phe).

cis-Phenothrin is more resistant than the trans isomer to hydrolysis at the ester linkage (Refs. 56 and 57) and greater amounts of the ester metabolites of this isomer were recovered.

In a more recent study, [1R, trans]- and [1R, cis]-phenothrin labelled at the benzylic carbon were orally administered to male rats at the rate of 2 mg/rat (ca. 10 mg kg^{-1}). During 2 days, 75% of the radiocarbon from trans phenothrin was recovered in the urine with 22% in the faeces, whereas with cis phenothrin 20% and 73% of the radiocarbon was excreted into the urine and the faeces, respectively. The major urinary metabolites of trans isomer was 4'-OH-PBacid sulfate amounting to 42% of the applied radiocarbon, followed by free PBacid (10%) and 4'-OH-PBacid (6%). The glucuronide and glycine conjugates of these acids were formed in smaller amounts.

The faecal metabolites of the trans isomer and urinary metabolites of the cis isomer were mostly hydrolysis products of phenothrin. In the faeces, the cis isomer gave rise to mainly ester metabolites in amounts varying from 2% (4'-OH,ωt-alc-c-phe) to 13% (4'-OH,ωt-acid, 2-OH (t)-c-phe) of the applied radiocarbon. These were oxidised at the 4'-position of the phenoxy group, one of the geminal dimethyls, and/or the methyl groups attached to the vinyl group of the acid moiety (Ref. 60).

In the original study (Ref. 58) 4'-OH-PBacid sulfate was not positively demonstrated. The striking difference could be due to the administered dose (200 mg kg^{-1} vs. 10 mg kg^{-1}), but it is also possible that the difference was due to decomposition of 4'-OH-PBacid sulfate during work-up. An additional experiment is desirable to confirm this point.

The metabolic pathways of phenothrin are presented in Figure 18.

The acid moiety of phenothrin isomers has never been studied with respect to in vivo metabolism. However, the metabolic fate of the acid moiety of bioresmethrin or [1R, trans]-5-benzyl-3-furylmethyl chrysanthemate gives the relevant information, since bioresmethrin readily yielded chrysanthemic acid (t-CA) in rats. After oral administration of [^{14}C-carboxy labelled] bioresmethrin, 73% of the radiocarbon was recovered during 6 days, 41% in the urine and 32% in the faeces. About half of the radioactivity was identified. the major metabolite was trans chrysanthemic dicarboxylic acid (ωt-acid-t-CA) in both urine and faeces, respesenting 13% of the applied radiocarbon, followed by cis chrysanthemic dicarboxylic acid (ωt-acid-c-CA). Two hydroxymethyl chrysanthemic acids (ωt-alc-t- and ωt-alc-c-CA) were also found. The aldehyde (ωt-ald-CA) is assumed to be the most likely intermediate for the epimerisation reaction (ωt-ald-t-CA ωt-ald-c-CA), but its presence was not positively demonstrated. Some of the t-CA, ωt-alc-CA and ωt-acid-CA were excreted as conjugates, which were cleaved by incubation with glucosulase (Figure 19) (Ref. 61).

4.2 Permethrin

The metabolism of [1R, trans]-, [1R, trans]-, [1R, cis]- and [1R, cis]-permethrin in male rats was carried out comprehensively using four radioactive preparations, namely [$Cl_2{}^{14}C$=CH-], [^{14}CO] of the acid moiety and [α-$^{14}CH_2$]-, [phenoxy-^{14}C] of the alcohol moiety. During 12 days after single adiministration, radiocarbon from both acid- and alcohol-labelled trans-permethrin was eliminated into the urine (70-82% of the dose) with relatively little of the radiocarbon appearing in the faeces (8-18%), expired gases or retained in the tissues. In contrast only 37-54% of the excreted radiocarbon from cis isomers appeared in the urine, with 39-47% in the faeces.

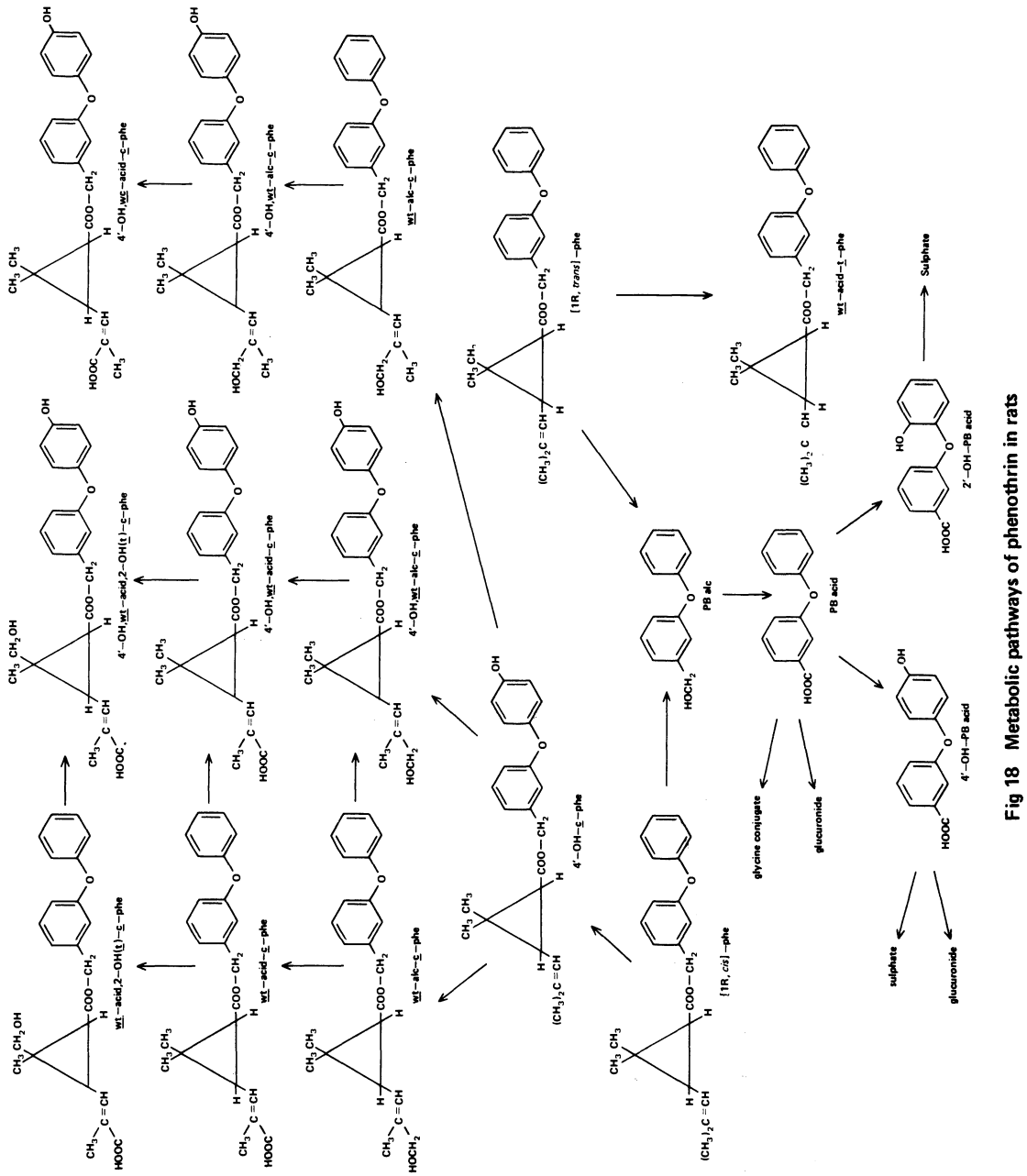


Fig 18 Metabolic pathways of phenothrin in rats

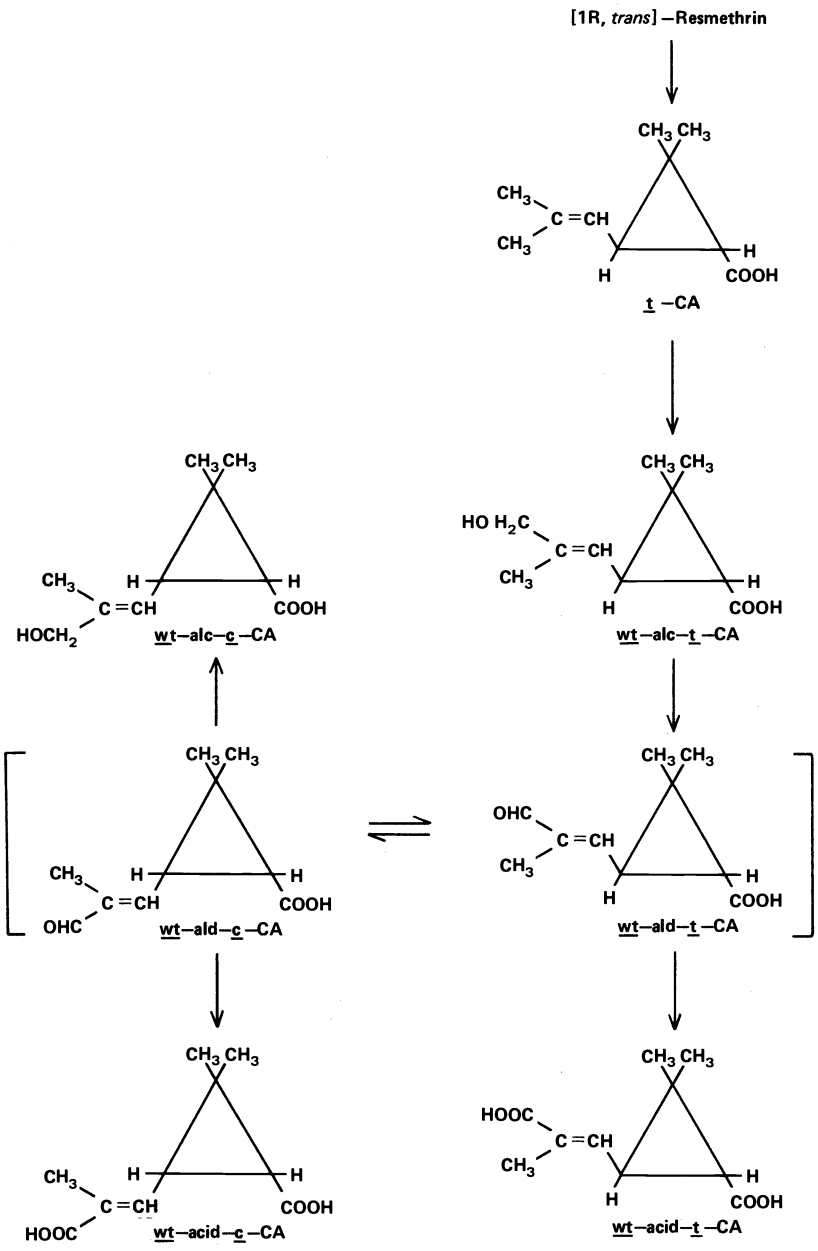


Fig 19 Proposed metabolic pathways of [1R, *trans*]—chrysanthemic acid in rats

The 24 hr-pooled urine and faeces (61-86% of the radioactivity depending on the isomers) were analysed for metabolites.

Unchanged permethrin was detected only in faeces, and accounted for 1.3-5.3% and 4.6-7.3% of the dose of trans and cis permethrin respectively. The faeces contained other ester metabolites of cis permethrin such as 2'-HO-per, 4'-HO-per, t-HO-per and 4'-HO, t-HO-per (see Figure 20 for abbreviations) each accounting for 1-5% of the administered radiocarbon. No ester metabolites of trans permethrin were excreted.

The major metabolite from the acid moiety was Cl_2CA, which was mostly excreted in urine, conjugated with glucuronic acid (gluc). This accounted for ca 50-63% of the dose from trans permethrin and 15-22% from cis permethrin.

Oxidation at either of the geminal dimethyl groups occurred to the extent of 4.3-10.4% (trans) or 12.2-14.9% (cis), and these oxidised Cl_2CA's were excreted in urine and faeces, as such or as the lactone or glucuronide.

The major metabolite from the alcohol moiety was 4'-HO-PBacid sulphate, accounting for 30.7-42.8% of the dose (trans) and 19.5-29.3% (cis). From cis permethrin 2'-HO-PBacid sulphate (ca. 3%) was identified. Another significant metabolite was PBacid. This occurred free and as glucuronide as glycine conjugates, and accounted for 25-31% (trans) and 5.7-10.1% (cis) of the dosed radiocarbon. Except for a trace of BPacid, all the above metabolites from the alcohol moiety were excreted entirely in the urine. The faeces of rats dosed with trans permethrin however contained 1-2% of the radioactive dose as PBalc.

Thus, substantial portions of the radioactive metabolites in the recovered excreta were identified. The proposed metabolic pathways for cis and trans permethrin are shown in Figure 20.

The metabolites discussed above were excreted within the first 24 hr following the treatment. However, the 24-48 and 48-72 hr samples of excreta were reported to give TLC patterns and proportions of metabolites very similar to those obtained with the 0-24 hr samples.

Based on the above results, there appears no significant metabolic difference between 1R and 1RS isomers, although cis permethrin isomers are apparently more liable to undergo oxidative metabolism than the trans counterparts (Refs. 62,63 and 64).

When four separate radioactive permethrin preparations ([1RS, trans]- and [1RS, cis]-labelled with [^{14}C] at either the CO of the acid or the CH_2 of the alcohol moiety) were administered orally, each to one lactating goat, at the rate of ca. 10 mg/kg daily for 10 consecutive days, excretion of the radiocarbon was rapid. Most radiocarbon had been eliminated from the previous treatment before the next dose had been administered. By the time of slaughter, 24 hr after the tenth treatment, 72-79% and 26-36% of the total radiocarbon of trans- and cis-permethrin respectively had been recovered in the urine. The faeces contained 12-15% and 52-68% of the radioactivity respectively.

The residue in the milk, during and after administration, was in the range of 0.03-0.09µg kg^{-1} and 0.01-0.03µg kg^{-1} (permethrin equivalents) for cis and trans isomers, respectively. In most tissues of goats, the radiocarbon was detected but none contained residues higher than 0.04 mg kg^{-1} for the trans isomers or 0.25 mg kg^{-1} for the cis isomer. The radiocarbon contents in the fat of goats receiving cis-permethrin were approximately 10 times higher than those of goats given the trans isomer.

No metabolites were resolved or identified in the study (Ref. 65).

A more detailed study of retention and metabolism in ruminant animals was conducted using three consecutive oral administrations of [1RS, trans]- or [1RS, cis]-permethrin [^{14}C]-labelled in the CO- or CH_2-positions to lactating Jersey cows at the daily dose of 0.9-1.1 mg kg^{-1}. The radiocarbon contents in the milk, during and after administration, were relatively low, with the maximum level of 200-250µg kg^{-1} permethrin equivalents (higher for cis that for trans) during dosing, dropping below 100µg kg^{-1} within 2-4 days after termination of the treatment. Most of the radiocarbon in milk was likely to be the unmetabolised trans and cis-permethrin and a small amount t-HO-per (cis).

Among the tissues of the animals slaughtered 12-13 days after initiation of the administration, fat and liver contained the highest residues with a maximum concentration of 210-335µg kg^{-1} equivalents. Most of the radioactivity in fat was due to the unmetabolised permethrin, and in liver some was due to the unidentified products.

39-47% and 52-57% of the radiocarbon of trans-permethrin was recovered in the urine and in the faeces during the whole experiment, while with cis-permethrin, 22-28% of the radiocarbon were excreted to the urine and 60-76% in the faeces.

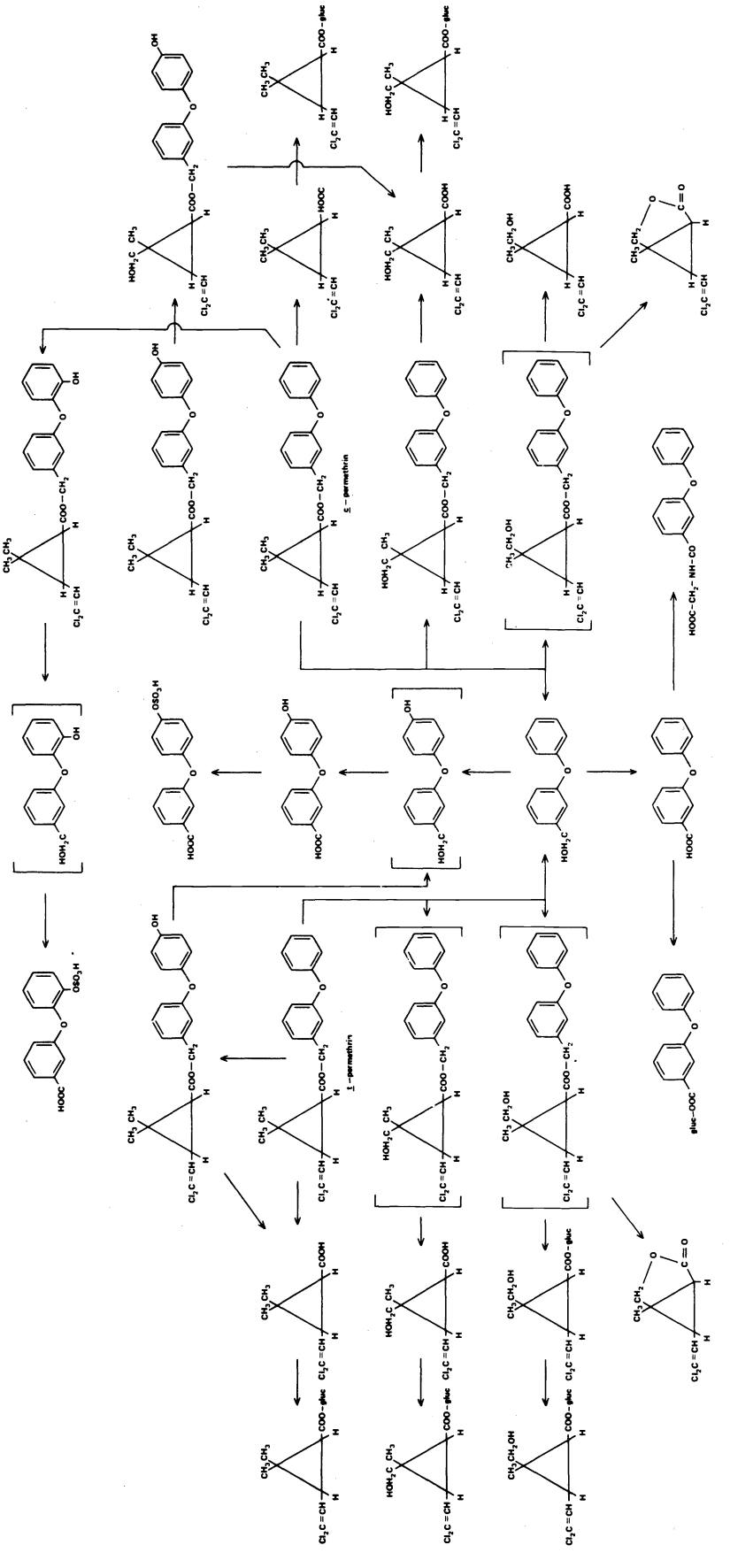


Fig 20 Metabolic pathways for [1 R, *trans*] – and [1 R, *cis*] –permethrin. These pathways are also applicable to [1 RS, *trans*] – and [1 RS, *cis*] –permethrin and to phenoxybenzyl alcohol and [1 R, *trans*] –Cl,CA. Additional pathways which are not shown yield several minor metabolites, most of these being formed from *cis*–permethrin.

In the inital 3-day pooled excreta, 38-39% of the radiocarbon was present as ester metabolites, with t-HO-per as the major product (14-22%), followed by unchanged permethrin, t-HO, 4'-HO-per, and t-HO-per glucuronide.

Among the metabolites of the acid moiety, comprising 54-56% of the radiocarbon, Cl_2CA, free and as glucuronide and c-HO-Cl_2CA and its lactone were found in the largest amounts. The major metabolites from the alcohol moiety were PBacid conjugated with glutamic acid and glycine, accounting for 15 (cis) to 39% (trans) of the recovered radiocarbon. PBalc equivalent to 8-10% of the radioactivity in the excreta was also present.

Thus, altogether 18 metabolites were identified from trans-permethrin and 15 from cis-permethrin. Compared with rats, cows excreted a larger portion of ester metabolites, and are unique in utilising glutamic acid for conjugation of carboxylic acid metabolites. They also carry out more extensive hydroxylation on the trans-methyl group and less on the phenoxy group; for example, 4'-HO-PBacid sulphate was a major metabolite in rats, but it was detected only in a trace amount in cows (Ref.s 64 & 66).

Four separate [^{14}C]-permethrin preparations, [1RS, trans] and [1RS, cis] each labelled at the CO or CH_2 group, were orally administered to laying hens at the rate of 10 mg kg^{-1} daily for three consecutive days. The radiocarbon was largely eliminated from the body within 1 day after the last dose, and 87-90% of the total radioactivity was recovered during 9 days, a small portion as $^{14}CO_2$ (0.7-4.7%).

The radioactive residues, in fat and skin on sacrifice at 10 days after initiating the treatment, were at most 0.66 and 0.1% of the administered dose. The trans-permethrin equivalent residues were one seventh to one tenth of the cis isomer. The radiocarbon was mainly due to the parent compounds. Other tissues were generally low in radiocarbon contents.

Eggs obtained 3 to 5 days after termination of the treatment contained highest radiocarbon residues. These were 2.5-3.0 mg kg^{-1} in yolk from cis and ca. 0.6 mg kg^{-1} from trans isomers, which were approximately 10 times higher than residues in white. In the yolk, unchanged permethrin was the only ester metabolite (ca. 0.3 mg kg^{-1}) from trans-permethrin, whereas from eggs of hens dosed with cis isomers, ester metabolites including t-HO-permethrin, whereas from eggs of hens dosed with cis isomer, ester metabolites including t-HO-permethrin as well as its suphate conjugate were found along with ca. 1.2 mg kg^{-1} of unmetabolised cis-permethrin. The remainder of the radiocarbon in yolk and white was a variety of metabolites also detected in the excreta.

After dosing cis permethrin, the following ester metabolites were identified in the excreta from the first 3 days of the treatment schedule, t-HO-per sulphate, t-HO-per, 4'-HO-t-HO-per (total amounts of 17% of the recovered radiocarbon were identified). No ester metabolites were derived from trans-permethrin. Unchanged permethrin accounted for 7.4% (trans) and 12.6% (cis) of the recovered radiocarbon.

From the acid moiety Cl_2CA, Cl_2CA-taurine, Cl_2CA-glucuronide, c-HO-Cl_2CA and its glucuronide, sulphate and lactone, t-HO-Cl_2CA were obtained. From the alcohol moiety PBalc as free and as glucuronide, 4'-HO-PBalc free and as the sulphate and another unidentified conjugate, PBacid, 4'-HO-PBacid and its sulphate and another conjugate were obtained. More ester cleavage metabolites were produced from trans-permethrin than from the cis isomer.

In hens, the permethrin isomers are not only hydrolysed, but they also undergo extensive oxidation as noted with rats and cows. 4'-Hydroxy- and trans-hydroxymethyl ester metabolites were detected in egg yolk and excreta from hens dosed with cis-permethrin, but not with trans-permethrin. This implies that if hydroxy derivatives of trans-permethrin are formed, they undergo rapid hydrolysis, and when cis-permethrin is hydroxylated at the cis-methyl group, it also is cleaved prior to excretion.

A similar situation exists relative to the excreta with rats whereas with cows both isomers are excreted in part as monohydroxy and dihydroxy esters. The preference in hydroxylation site, based on the identified metabolites is the same with hens and rats, i.e., phenoxy>cis methyl>trans methyl with trans-permethrin, and phenoxy>trans methyl>cis methyl with cis-permethrin. In contrast, with cows both trans- and cis-permethrin have the same order of preference of trans methyl>cis methyl = phenoxy. Metabolites detected with hens but not with rats or cows were the cis isomer of t-HO-per sulphate, the trans isomer of c-HO-Cl_2CA sulphate, 4'-HO-PBalc sulphate and Cl_2CA taurine conjugate.

Both hens and rats extensively utilised glucuronic acid and sulphate conjugates in excretion of permethrin metabolites, but hens formed the taurine conjugate and several unidentified conjugates, possibly with conjugating moieties not utilised by rats or cows (Ref. 67).

4.3 Cypermethrin

Although Hutson (Ref. 56) states that the in vivo fate of cypermethrin is similar to that of permethrin in terms of rates of excretion, routes of excretion, tissue residues, and the structure of the excreted metabolites, no detailed results appear yet in the open literature.

The [1RS]-cis- and -trans-cypermethrin [^{14}C] labelled at the α carbon of the benzyl ring when orally administered to mice was apparently rapidly eliminated, 27% and 55-67% of the dosed radiocarbon being recovered in the urine during 24 hr. Among the urinary metabolites N-(3-phenoxybenzoyl) taurine was identified, accounting for 9% and 13-23% of the dosed cis- and trans-cypermethrin. No other mouse metabolites have been reported (Ref. 68).

Under similar conditions, no such taurine conjugate was formed in rats dosed with trans-cypermethrin.

4.4 Deltamethrin

On oral administration of deltamethrin ([1R cis, S] [^{14}C] labelled at the αC or in the Br_2C=CH group) to rats, the radiocarbon was rapidly and almost completely eliminated from the body. After 8 days post treatment, carcass and tissues contained 1-2% of the dosed radiocarbon. With [^{14}CN]-labelled compound, approximately 20% of the radiocarbon remained in the animal body after 8 days, the highest concentrations being in skin and stomach. Unlike the fenvalerate experiments, no radiocarbon was determined in hair. Essentially all the radiocarbon in the stomach was thiocyanate. No noticeable $^{14}CO_2$ was obtained with either radioactive preparation, although CN from fenvalerate yielded CO_2, as will be described below.

The major faecal residue was intact deltamethrin, accounting for 13-21% of the dosed radiocarbon, followed by 4'-HO- and 5-HO-deltamethrin and a trace amount of 2'-HO-deltamethrin. Intact deltamethrin and the 4'-HO-derivative appeared not only as the administered αS-epimer, but also in parts as the αR-epimer, probably due to artifactual racemisation on exchange of the α-proton in methanol.

The metabolites from the acid moiety were mostly Br_2CA, free (10% of the dosed radiocarbon) and conjugated with glucuronic acid (51%), with trace levels (less than 1% each) of the glycine conjugate of Br_2CA, free and the glucuronide conjugate of the hydroxy acid probably on the methyl group trans to the carboxyl.

The major metabolites of the aromatic portion of the alcohol moiety were PBacid derivatives (20% of the administered radiocarbon) and 4'-hydroxy derivatives (55%). 4'-HO-PBacid sulphate accounted for ca. 50% of the α[^{14}C] labelling free and with small amounts of the glucuronide. PBacid was excreted either without conjugation (13%) or as the glucuronide and glycine conjugate.

The CN group was converted mainly to thiocyanate and a small amount of 2-iminothiazolidin-4-carboxylic acid (ITCA) (Ref. 69).

Oral administration to male mice of the above alcohol-labelled or acid-labelled deltamethrin resulted in the complete recovery (ca. 99%) of the radiocarbon in the excreta after 8 days with 57-65% in the urine and 34-42% in the faeces. The recovery of the cyano-labelled deltamethrin was lower, 93%, with 35.5% in the urine and 58% in the faeces, with the remaining radioactivity in the skin and stomach. No expired radioactive carbon dioxide was detected. Smaller amounts of unmetabolised deltamethrin were excreted in faeces in mice than in rats.

The faeces but not the urine contained four monohydroxy ester metabolites (2'-HO-, 4'-HO-, 5-HO-, and t-HO-deltamethrin) and one dihydroxy metabolite (4'-HO-t-HO- deltamethrin). Two unidentified ester metabolites were also detected in trace amounts.

Major deltamethrin metabolites from the acid moiety were Br_2CA, t-HO-Br_2CA and their conjugates. As compared with rats, much larger amounts of t-HO-Br_2CA and its conjugates were formed in mice.

A major alcohol moiety metabolite was the taurine conjugate of PBacid in the urine which was undetectable in rats. Again as compared with rats, more free PBacid as well as 4'-HO-PBacid and less 4'-HO-PBacid sulphate were excreted in mice. Although not included, in Figure 21 below, a trace amounts (ca. 1% each) of 3-phenoxybenzyl alcohol and 3-phenoxybenzaldehyde was also detected.

In mice, no ITCA was found (Ref. 70).

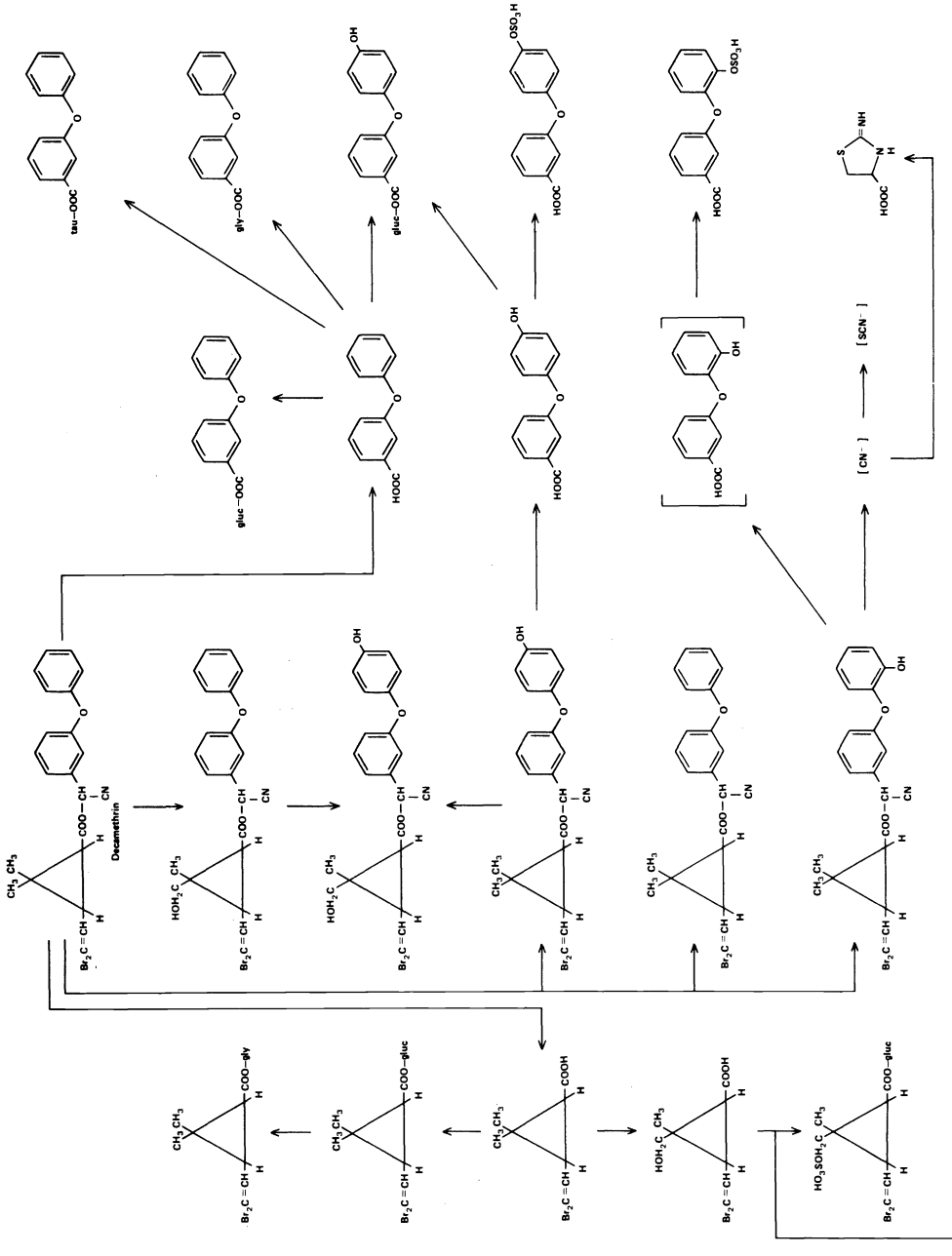


Fig 21 Metabolic pathways of decamethrin in rats and mice

Figure 21 shows the metabolic pathways of deltamethrin in rats and mice.

4.5 Fenpropathrin

The metabolism of [αRS]-fenpropathrin was studied in rats using two [^{14}C]-labelled (benzyl-ring and cyclopropyl) samples. Excretion of orally administered benzyl radiocarbon was rapid in both sexes (approximately 97% in 48 hr), 57% of the dose being recovered in the urine and 40% in the faeces. No sex difference was observed in the excretion pattern. Only 1.5% of the administered radioactivity remained in the animals 8 days after treatment. No significant amounts of radiocarbon were detected in the expired air.

In the 24 hr pooled faeces containing 16% of the administered radioactivity, unchanged fenpropathrin, 4'-HO-fenpropathrin, α-cyano-3-phenoxybenzyl-trans-2-hydroxymethyl 2,3,3-trimethylcyclopropane carboxylate and 4'-HO-PBacid were major metabolites.

Among urinary metabolites from the alcohol moiety, the major one (90% of the radiocarbon) in the 24 hr urine was 4'-HO-PBacid sulphate. Three other metabolites were indentified as 4'-HO-PBacid, PBacid and its glycine conjugate.

The major urinary metabolites from the acid moiety were 2,2,3,3-tetramethylcyclopropane carboxylic acid conjugated with glucuronic acid and the 2-hydroxymethyl analog. These amounted to 50% and 21% of the dosed radioactivity, respectively (Ref. 71).

4.6 Fenvalerate

Orally administered radiolabelled fenvalerate-[2RS, αRS] was rapidly metabolised in rats and mice, and the acid and the alcohol fragments except the CN group were eliminated substantially completely from the body of either sex of both species with a half-life of 0.5-0.6 days. The radiocarbon from the ^{14}CN-labelled fenvalerate was slowly excreted with a half-life of 1.7-2 days in rats and 1-1.2 days in mice. Recovery of total radiocarbon in faeces and urine 6 days after administration, was 93-100% in both animal species with the acid [^{14}CO]- and the alcohol [^{14}CH]-labelled compounds with no detectable radiocarbon in expired gases. With the CN-labelled fenvalerate, 89% and 75-81% of the radiocarbon was recovered in mice and rats respectively, approximately 6-13% being detected in the expired air. The excretion pattern of five consecutive dosings of fenvalerate was similar in rats and mice.

Tissue residues, 6 days after single administration of the acid and the alcohol labelled compounds, were very low in both species. With the ^{14}CN-labelled compound, in contrast, somewhat higher radiocarbon residues were found in hair, skin and stomach contents. These were similar to the tissue distribution of radioactive potassium cyanide or potassium thiocyanate separately administered to the animals. The radiocarbon in the stomach contents was mainly thiocyanate. Residue levels of the CN-labelled fenvalerate in these tissues were lower in mice than in rats. Faeces from rats and mice contained unmetabolised fenvalerate accounting for 8-20% of the dosed radiocarbon, and two ester metabolites, 2'-HO- and 4'-HO-fenvalerate. The major urinary metabolite from the acid labelling in 2-day pooled urine amounted to 50-55% of the dosed radioactivity and was 2-(4-chlorophenyl) isovaleric acid [Cl-Vacid in Figure 22]. The glucuronide conjugates accounted for 28-36% of the dose in rats and 16-23% in mice. 2-(4-Chlorophenyl)-3-hydroxymethylbutyric acid [3-HO-Cl-Vacid] was the second major metabolite in mice (10-13% of the dosed radiocarbon), while in rats the amounts were smaller. Among minor products identified or presumed were 2-(4-chloro-phenyl)-3-methylbutan-4-olide [3-HO-Cl-Vacid lactone], 2-(4-chlorophenyl)-2-hydroxy-3-methyl butan-4-olide [2,3-HO Cl-Vacid lactone] free and conjugated, 2-(4-chlorophenyl)-3-methyl-2-butan-4-olide [Cl-Bacid lactone], 2-(4-chlorophenyl)-cis-2-butene-dioic anhydride [Cl-BDacid anhydride] and 2-(4-chlorophenyl)-2-hydroxy-3-hydroxymethylbutyric acid [2,3-HO-Cl-Vacid].

In rats receiving [^{14}CH]-fenvalerate, the 2-day pooled urine contained 62-63% of the dosed radioactivity. The major metabolite was 4'-HO-PBacid and its sulphate with ca. 2-3% and 40% of the dosed radiocarbon. In contrast, in mouse urine which contained 63-64% of the radiocarbon, the amounts of these two metabolites were less (10-27%). PBacid and its derivatives including glycine, glucuronide and taurine conjugates were obtained in relatively large amounts. The taurine conjugates representing 10-13% of the dose were not detected in rat urine. Some 2'-HO-PBacid was excreted as sulphate from rats but not from mice.

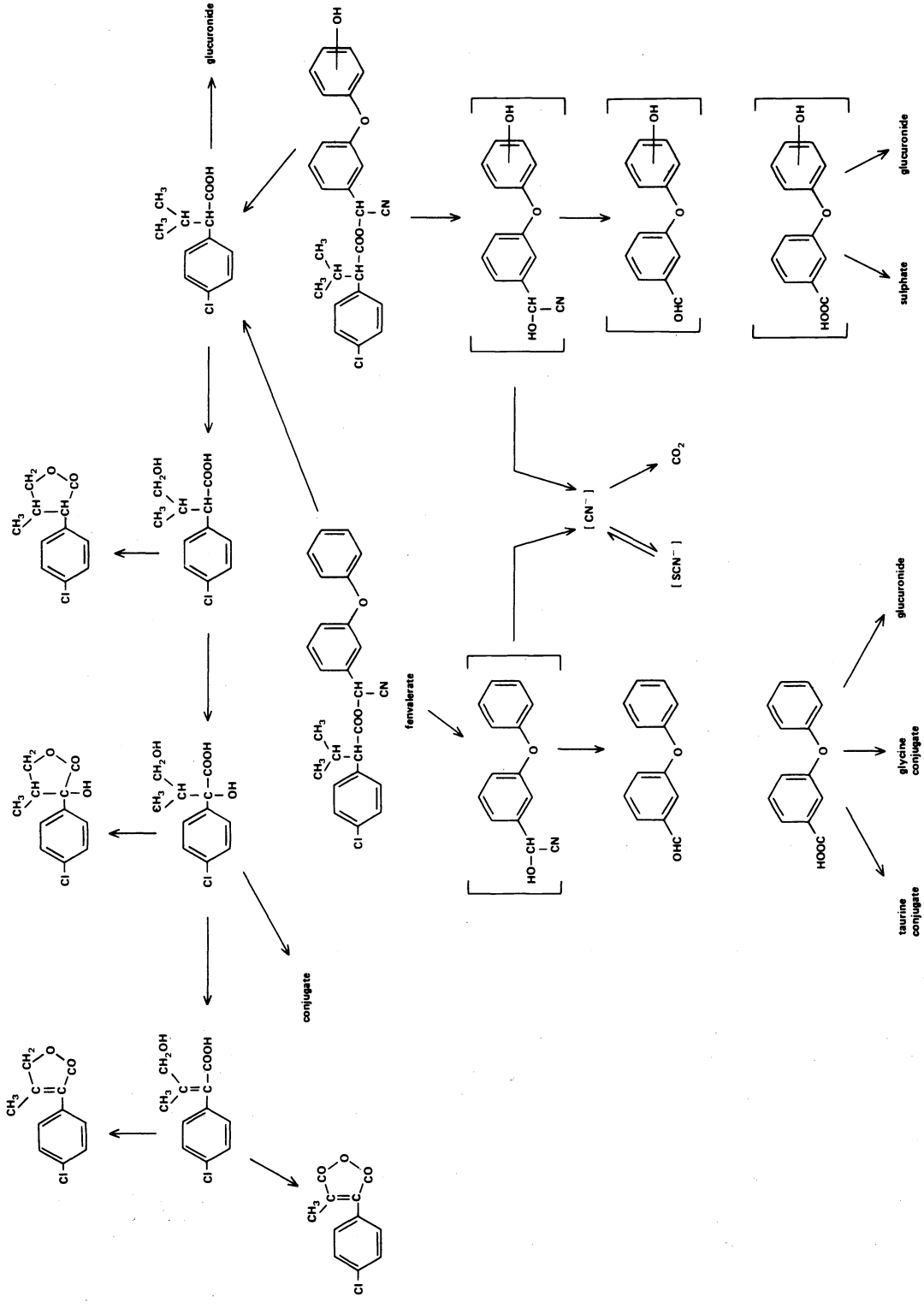
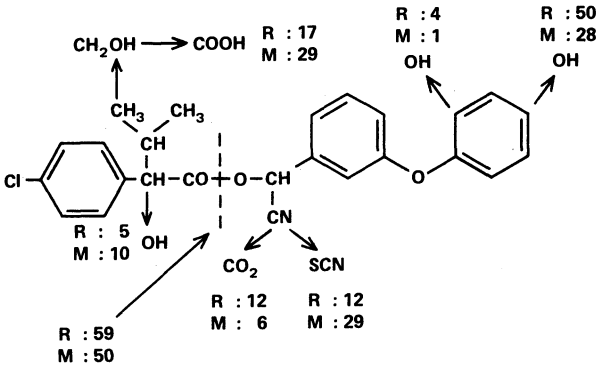


Fig 22 Proposed metabolic pathways of fenvalerate in rats and mice



Site of metabolic attack of fenvalerate in rats (R) and mice (M) (% of dose)

With [^{14}CN]-fenvalerate, the major urinary metabolite in both species was thiocyanate, which was recovered to a greater extent in mice as compared with rats, based on the results for 2-day pooled excreta. No ITCA was positively demonstrated in either of the species. The amounts of the expired radioactive carbon dioxide was slightly higher in rats than in mice.

The metabolic pathways for fenvalerate in rats and mice are presented in Fig. 22.

Thus there were some apparent species differences in the nature and amount of the metabolites in excreta as summarised in the above figure. However, no significant sex difference was seen in either species.

The preliminary studies revealed that the metabolism of the resolved isomers of fenvalerate ([2S, ±RS] and [2S, ±S) proceeds apparently similarly to racemic fenvalerate in these two animal species (Refs. 72 & 73).

5 RESIDUE ANALYSIS PROCEDURES

5.1 Phenothrin (Ref. 74)

Residue determination of intact phenothrin by gas-liquid chromatography (GC) appears difficult due to its poor sensitivity to the GC detector commonly used in residue analysis, though some residue data of d-phenothrin (mixture of [1R, trans] and [1R, cis] isomers) on stored sorghum (0.4-0.95mg kg^{-1}) were obtained by GC equipped with a flame ionisation detector (Ref. 75). Kato et al (Ref. 76) successfully applied combined gas-liquid chromatography/mass spectrometry (GCMS) to determine intact d-phenothrin residues in blood. Selected ion monitoring of d-phenothrin gave as minimum detectable amounts 0.1ng and 0.04ng at mass ions, m/z 350 (parent) and 183, respectively, thereby allowing confirmation as well as quantification at 0.005mg kg^{-1} or even less. Desmarchelier (Ref. 77) also briefly reported the value of GCMS for analysis of phenothrin in stored grains at residue levels below 0.01 mg kg^{-1} without presenting detailed data.

As a determination procedure, Simpson (Ref. 78) adopted high pressure liquid chromatography in combination with clean up by partitioning between n-hexane and acetonitrile, and alumina column chromatography. By this method, phenothrin residues of 1.56-2.72 mg kg^{-1} were quantified in stored sorghum, though data on detection limits and recoveries are not presented (Refs. 78 & 79).

Derivatisation methods have been more commonly adopted for residue analysis. Takimoto et al (Ref. 80) analysed phenothrin residues by the procedures which included potassium hydroxide-catalysed hydrolysis of phenothrin in aqueous methanol and subsequent pyridine-catalysed esterification of the liberated alcohol moiety with 2,4-dichlorobenzoyl chloride. When analysed by GC fitted with a 0.8m long glass column packed with either 4% FFAP on Gas-chrom

Q or with 3% OV-101/3% Apiezon Grease L on Gas-chrom Q, 3-phenoxybenzyl-2,4-dichlorobenzoate showed excellent sentisitivity, i.e., 0.04-0.1ng as minimum detectability to an electron capture detector which permitted detection of phenothrin at 0.005-0.02 mg kg^{-1}.

Desmarchelier (Ref.s 77 and 81) extended the colourimetric procedures which had originally been devised by McClellan (Ref. 83) for residue analysis of pyrethrum. The procedures were based on a colour reaction of a modified Deniges reagent with chrysanthemic acid formed by hydrolysis of phenothrin using sodium hydroxide in aqueous alcoholic solution. Limits of detection are reported to be 0.1-0.2mg kg^{-1}. The method appears less laborious but less selective and less sensitive than that reported by Takimoto et al (Ref. 80). In addition, a significant difference in extinction coefficient observed at 584nm between (±)-cis and (±)-trans chrysanthemic acid necessitates prior separation of cis and trans isomers for accurate determination of phenothrin.

Thin-layer chromatography (TLC) based on the above colour reaction has also been applied as a semiquantitative procedure (Refs. 77 and 81).

The solvent or solvent combinations, selected for extraction of phenothrin, depend upon the nature of substrates. From the substrates with high-moisture contents like cabbage and green pepper, phenothrin was effectively extracted by chopping and blending them either with polar solvents, e.g. methanol, acetonitrile, or with nonpolar-polar solvent mixtures e.g. benzene-ethanol (Ref. 80). For aged phenothrin residues on cooked rice, 24-hour extraction with polar solvents, e.g. methanol and ethanol, gave complete recoveries whereas extraction with nonpolar solvents e.g. light petroleum and hexane, resulted in poor recoveries (Ref. 82). From dry and low moisture substrates including rice, barley, wheat, oats and sorghum, phenothrin is efficiently extracted with either polar, nonpolar or dual solvent systems. Hulled rice grain was ground and macerated overnight in methanol (Ref. 80). Wheat and barley were soaked overnight as whole grain either in light petroleum or in ethanol (Ref. 82). Desmarchelier (Ref. 81) reported that use of light petroleum simplified cleanup in the analysis of grains.

As cleanup procedures, both liquid chromatography (LC; Florisil and alumina) and TLC (silica gel) are used for intact phenothrin residues (Refs. 78 and 80). The Florisil column chromatography coupled with benzene-ethyl acetate extraction resulted in 93% recovery, as determined by GCMS, from blood fortified at 0.05 mg kg^{-1} (Ref. 76). Cleanup procedures employed for the derivatisation methods obviously reflect the nature of the derivatives as well as substrates. The method of converting phenothrin to 3-phenoxybenzyl 2,4-dichlorobenzoate which provided 81-88% recoveries for cabbage, green pepper and hulled rice fortified at 0.1-1 mg kg^{-1} included cleanup by either LC or TLC before both hydrolysis and GLC quantification (Ref. 80). The colourimetric method employed partition cleanup between aqueous phases and organic solvents, e.g., light petroleum or chloroform, and satisfactory recoveries of 87-91% were achieved for cooked rice.

5.2 Permethrin

Analytical procedures published in the open literature for the determination of residues of permethrin have largely involved gas-liquid chromatographic methods for measurements of the intact ester (Refs 84-93, 94, and 95). Most methods follow very similar procedures involving:

a) initial extraction into organic solvent,

b) liquid-liquid partitions to remove water soluble coextractives and/or lipid coextractives,

c) cleanup of the extracts by adsorption chromatography,

d) quantitative determination of the insecticide residue by gas-liquid chromatographic techniques.

A summary of the methodology used by various workers for each of the steps outlined above is given in Table 1.

In general a hydrocarbon solvent, e.g., hexane either alone or in combination with a more polar solvent, e.g., acetone, was used for the initial extraction of the pyrethroid. An exception is given by Fujie and Fullmer (Ref. 89) who used 9:1 methanol:water for the extraction of soil residues. George et al (Ref. 86) used dichloromethane to extract the compound from treated cabbage and green pea samples. No references to extractability studies for weathered residues of permethrin were found. However, Chapman and Harris

(Ref 91) (using fortified samples) investigated the relative percentages extracted by an initial acetone extraction (60-90% recovered) and subsequent acetone wash of the extracted plant fibre (10-30% recovered).

Zitko et al (Ref. 96) made use of a small Amberlite XAD-2 resin column to extract residues from seawater.

The use of a partition between the organic extracts and an aqueous system to remove water soluble coextractives and the polar extraction solvent was favoured by most workers, although a variety of different systems, e.g., water-petroleum-ether, saturated sodium chloride-ethyl acetate, were employed. No justifications for the selection of particular solvent systems were given. The removal of lipid coextractives, where necessary, by the use of a pentane:acetonitrile partition followed by dilution of the acetonitrile extracts with water and subsequent back partition into pentane, is described by Simonaitis and Cail (Ref. 87). A modification of the above procedure which uses hexane and dimethylformamide has been employed in order to take advantage of the highly favourable partition of permethrin between these solvents (Ref. 96).

Gel permeation chromatography (GPC) was reported by Fujie and Fullmer (Ref. 89) to be an efficient tool for pesticide - lipid separation in permethrin residue analysis. Excellent pesticide - lipid separation was achieved by using Bio-Beads S-X3 and a hexane-ethyl acetate solvent system.

Oehler (Ref. 95) has described the use of high pressure liquid chromatography (HPLC) using a bonded phase column as a further alternative for the removal of coextracted lipid from bovine tissue extracts.

Removal of interfering substances by adsorption chromatography was carried out using Florisil, alumina or silica gel, either individually, in tandem, or even as a mixture of all three plus cellulose and Nuchar. Permethrin was usually eluted from the columns using only very slightly polar solvents, e.g., benzene or 1.5% diethyl ether in pentane. Williams reported that low limits of determination $<0.1 mg\ kg^{-1}$ were not obtainable on bean leaves using the five component mixed column referred to previously. Chapman and Harris (Ref. 91) reported a high percentage recovery of permethrin from a variety of adsorption columns using different solvent eluate systems. They concluded that the use of Florisil with benzene-hexane (80:20) elution, following a pre-wash with hexane, afforded good cleanup for most crops studied. It was stated that the use of acetone in hexane or chloroform in hexane in efforts to reduce the use of benzene in the laboratory could only be at the expense of adequate cleanup. No more acceptable aromatic substitute for benzene, e.g., toluene, was studied however. Low limits of determination, $<0.05\ mg\ kg^{-1}$ were reported for a wide range of crops by Fujie and Fullmer (Ref. 89) using Florisil and elution with 10% diethyl ether in hexane.

Gas-liquid chromatography of permethrin was used to measure the total ester as a single peak when it eluted from columns of low loadings (3-5%) of a non polar methyl silicone stationary phase e.g, OV-101 or SE-30. Alternatively, semi-polar trifluoropropyl (QF-1, OV-210) or cyanopropyl silicone (OV-255, SP-2330) stationary phases at low loadings (1-6%) were used to separate the cis and trans isomers of the pyrethroid. Detection was usually by means of an electron capture detector, the lowest reported value for measureable amounts of the pesticide being ~10 pg. Chapman and Simmons (Ref. 92) examined the relative electron capturing responses of the cis and trans isomers of permethrin and concluded that they were identical. Hence the use of a total isomer GC measurement would not be significantly different for residues of varying isomeric composition. Chiba (Ref. 88), however, contradicts this observations as he reported that the trans isomer of permethrin has a 10% greater electron capturing response than the cis isomer. Simonaitis (Ref. 87) favoured the use of a flame ionisation detector for the high residues found after stored product treatment and reported linearity of detection over the range 15 to 485 ng injected.

Apart from the intact ester residue determinations, only three other publications, describing alternative residue methodology for permethrin are reported. Two techniques involve an initial hydrolysis of the extracted pyrethroid ester by refluxing in sodium hydroxide solution. George et al . (Ref. 86), adapted a procedure they had previously reported for the analysis of residues of bioethanomethrin (Ref. 98). Following hydrolysis, the resultant 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzyl alcohol were separated by acid-base partition. The Cl_2CA was converted to the trichloroethyl ester using trichloroethanol and a coupling agent, dicyclohexylcarbodi-imide. The PBalc was converted to a trichloroacetate using trichloroacetyl chloride. Following formation of the chlorinated esters, the residues were quantitatively determined using gas liquid chromatography with an electron capture detector. Recoveries from the procedure were 80-84% and sensitivity reported being 10 fold greater than the intact ester determination described in the same paper.

TABLE 1. Permethrin - GC residue analytical methods for intact ester

SUBSTRATE	EXTRACTION SOLVENT	PARTITION	CLEAN UP Column	CLEAN UP Elution	GAS LIQUID CHROMATOGRAPHY detector, column, temp, carrier gas, flow rate.	RETENTION TIME	LIMIT OF DETERMINATION or *smallest residue measured	%RECOVERY (fortification range)	
1) Potato 2) Bean leaves	acetone followed by acetone, hexane	saturated NaCl:ethyl acetate	1:1:1 mixture of alumina: Florisil silica gel and 5:2 cellulose: Nuchar	benzene	electron capture, 1.2m 3% SE30 at 190°C, 70ml min^{-1}	permethrin 2.6 mins	1) 0.005 mg kg^{-1} 2) 0.1 mg kg^{-1}	1) 94% (0.01-1.0 mg kg^{-1}) 2) 80% 0.1 mg kg^{-1}	Reference 84
Spring pasture grass	3:2 petroleum ether: acetone	water: pet ether	-	-	electron capture, 3% SE30 at 230°C.	not given	* 2 mg kg^{-1}	90%	Reference 85
Cabbage Green peas	dichloro-methane	-	a. silica gel b. aluminium oxide	dichloro-methane 1.5% ether in pentane	electron capture, no conditions specified	not given	* 0.04 mg kg^{-1}	102% (0.04-2.0)	Reference 86
Corn, cornmeal, flour, wheat	pentane	pentane: acetonitrile	alumina	3% ethyl acetate in pentane	flame ionisation, 1.2m 5% OV225 at 250°C, 30ml min^{-1}	cis isomer 9.5 mins trans isomer 10.0 mins	0.05 mg kg^{-1}	88-105%	Reference 87
Peach foliage	benzene	-	-	-	electron capture, 5% QF1 at 180°C, 35ml min^{-1}	cis isomer 3.7 mins trans isomer 4.5 mins	0.01 mg kg^{-1}	95%	Reference 88

TABLE 1. Cont'd.

SUBSTRATE	EXTRACTION SOLVENT	PARTITION	CLEAN UP Column	CLEAN UP Elution	GAS LIQUID CHROMATOGRAPHY detector, column, temp, carrier gas, flow rate.	RETENTION TIME	LIMIT OF DETERMINATION or *smallest residue measured	%RECOVERY (fortification range)	
1) Low moisture crops eg soya bean, cotton	1) hexane	-	a) gel permeation Bio beads SX-3 and/or b) Florisil	a) 1:3 hexane: ethyl acetate	electron capture, 1.8m SP2330 at 215°C 20ml min^{-1}. or	cis isomer 6 mins trans isomer 7 mins	-	64-112%	Reference 89
2) High moisture crops eg lettuce, cole	2) 2:1 hexane: isopropanol	-		b) 9:1 hexane: diethyl ether	Coulson conductivity detector, 1.2m 6% SP2330 at 215°C, 100ml min^{-1}.	cis isomer 5 mins trans isomer 6 mins	-	(0.05-5.0 mg kg^{-1})	
3) Soils	3) 9:1 methanol: water	3) aqueous: dichloro-methane							
Soils (organic pH4-silt loam pH8)	1:1 acetone hexane	sat. NaCl: hexane	mixture of alumina, silica gel and cellulose: Nuchar (as Ref 84)	benzene	electron capture, 1.8m 3% SP2330 at 220°C, 65 ml min^{-1}.	not given	not given	not given	Reference 90
Carrots, tomatoes, celery, onions and tobacco	acetone	aqueous acetone: hexane	Florisil 8.5% moisture	4:1 hexane: benzene	electron capture, 1.2m, 5% OV101 at 230°C. or 0.6m 10% QF1 at 210°C.	total permethrin 6.3 mins cis isomer 3.3 mins trans isomer 3.9 min	not given	99% (0.5-5.0)	Reference 91 (describes extraction and clean-up) Reference 92 (describe gas liquid chromatography)
Apples	30% acetone hexane	-	-	-	electron capture 9m 3% OV17+0.02% epicote 220°C. 30ml min^{-1}.	3.5 mins	0.1 mg kg^{-1}	95-100% (1-5 mg kg^{-1})	Reference 93

TABLE 1. Cont'd.

SUBSTRATE	EXTRACTION SOLVENT	PARTITION	CLEAN UP		GAS LIQUID CHROMATOGRAPHY detector, column, temp, carrier gas, flow rate.	RETENTION TIME	LIMIT OF DETERMINATION or *smallest residue measured	%RECOVERY (fortification range)	
			Column	Elution					
Cotton foliage (dislodgable residues only)	hexane	-	-	-	electron capture, 0.71m 3% SP2330 at 195°C.	-	0.1µg cm^{-2} on leaf surface	-	Reference 94
Bovine tissues	hexane	HPLC µPorogel	Florisil 20% dichlor-omethane in hexane	- -	electron capture, 1.8m 3% QF1 at 205°C.	cis 5.5 mins trans 8.0 mins	- -	92-106%	Reference 95
Seawater	XAD-2	-	-	-	electron capture 1.8m 4% SC30 at 215°C.	-	-	-	Reference 96

Desmarchelier (Ref. 81) reported on the determination of residues of permethrin on grains using a colourimetric procedure. Again the permethrin residue was hydrolysed and following acid-base partition a UV-visible complex was formed by the action of a modified Deniges reagent with the dichlorovinylcyclopropane carboxylic acid. Monitoring the transient UV-absorbance at 415 nm, either directly in solution or following TLC separation of the acid, allowed residues of 1.0 mg kg^{-1} to be measured.

The determination of [R] and [S]-epimers at C-1 in residues of permethrin has been described by Chapman and Harris (Ref. 99). Treatment of residual amounts of permethrin with optically pure sodium-l-menthylate at room temperature produced l-menthyl esters by transesterification. The enantiomeric forms of cis- and trans-permethrin-l-menthyl esters were separated and quantitatively measured by gas-liquid chromatography at levels down to 0.05 mg kg^{-1} in soil.

5.3 Cypermethrin

Reported residue analytical methods for the determination of cypermethrin are essentially the same as those previously cited in the review of permethrin residue methods. Lauren and Henzell (Ref. 85) used their procedure described for permethrin to measure cypermethrin residues on spring pasture grass. Chapman et al (Ref. 91) similarly adapted their permethrin procedure for the analysis of cypermethrin. They reported that alumina and aluminium oxide were not suitable adsorbants for cleanup of cypermethrin due to non-quantitative recovery of pesticide. Under the non-polar GC conditions described by them (see Table 1) cypermethrin eluted as a single peak (R_T 8.5 mins). Complete separation of the 4 geometrical isomers of cypermethrin could not however be obtained from the semi-polar column used to separate cis- and trans-permethrin. Three peaks were observed (R_T 8.9, 10.0, 11.0) when cypermethrin was chromatographed on this column and the workers reported that no improvement upon this partial resolution could be obtained from a range of phases investigated.

Residue analytical methods for the determination of cypermethrin in crops and soil down to 0.01 mg kg^{-1} are available (Ref.s 99 and 100). Similarly to the above procedures, cypermethrin residues were extracted by maceration (crops) or end-over-end tumbling (soils) with petroleum spirit:acetone mixture in the presence of anhydrous sodium sulphate. Following an aqueous wash to remove acetone, the extracts were cleaned up using liquid-solid adsorption chromatography using Florisil. Cypermethrin residues were subsequently quantitatively determined using GC with electron capture detection, either a) with almost no resolution of isomers using a 2% OV101 column or b) with partial resolution of isomers using a 2% OV225 column. To avoid breakdown of the pyrethroid during GC it was specified that no glass wool plug should be used at the injection zone of the column. Confirmation of cypermethrin residues was achieved by thin layer chromatography of the final sample extract followed by further GC.

Chapman and Harris (Ref. 99) modified their procedure for determination of permethrin optical isomers to accommodate cypermethrin. They found it necessary to form the benzyl esters initially and then transesterify to the l-menthyl esters. Subsequently the optically active forms of cis- and trans-cypermethrin were quantitatively measured by GC down to 0.05 mg kg^{-1} in soil.

As cypermethrin shares the common 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxyl moiety with permethrin then the procedures described by George et al (Ref. 86) and Desmarchelier (Ref. 81) involving measurement of the acid moiety should be applicable to the analysis of cypermethrin residues.

5.4 Deltamethrin

Two publications covering deltamethrin residue analysis are reported in the literature. Mestres et al (Ref. 102) have described a procedure for determination of the intact pyrethroid ester. The procedure is essentially similar to those used for permethrin analysis. Table 2 summarises the particular aspects of the methodology used for deltamethrin. The method is reported to be very sensitive, with a limit of determination of 0.005 mg kg^{-1} whilst residues as low as 0.001 mg kg^{-1} could be "detected".

In addition, Mestres et al (Ref. 102) described two techniques for the confirmation of deltamethrin residues. One such technique involves the use of a gas chromatograph-mass spectrometer operated in the 'selected ion monitoring' mode. The purified deltamethrin residues from their analytical procedure are qualitatively and quantitatively confirmed by monitoring the electron impact mass spectral fragments of m/z 181 and m/z 253 arising from the phenoxybenzyl and dibromovinylcyclopropane carboxyl moieties respectively. The second confirmatory procedure described is essentially that given in a separate paper by Hascoet and Landres (Ref. 103). This method involves an initial extraction and cleanup of the

TABLE 2. Decamethrin GC methods

SUBSTRATE	EXTRACTION SOLVENT	PARTITION	CLEAN UP Column	CLEAN UP Elution	GAS LIQUID CHROMATOGRAPHY detector, column, temp, flow rate.	RETENTION TIME	LIMIT OF DETERMINATION or *smallest residue measured	%RECOVERY (fortification range)	
1) Apples Peaches Grapes Tomatoes	1) acetonitrile	1) aqueous acetonitrile:pet: ether	1) Florisil 5% moisture or	1) 4:1 hexane: diethyl ether	electron capture 1.2m DC200, OV1 or OV101, 245°C.	10-12 mins	0.005 mg kg^{-1}	85-100% (0.02-0.1 mg kg^{-1})	Reference 102
2) Figs Olives Cotton-seed Cocoa berries Coffee beans	2) petroleum ether:diethyl ether	2) pet:ether: dimethyl sulphoxide	2) alumina	2) pet:ether					

TABLE 3. Fenvalerate GC methods

SUBSTRATE	EXTRACTION SOLVENT	PARTITION	CLEAN UP Column	CLEAN UP Elution	GAS LIQUID CHROMATOGRAPHY detector, column, temp, flow rate.	RETENTION TIME	LIMIT OF DETERMINATION or *smallest residue measured	%RECOVERY (fortification range)	
Cabbage Lettuce	acetonitrile	aqueous acetonitrile	Florisil	benzene: hexane	electron capture 1.8m 15% OV101, 225°C.	[RS] [SR] isomers 28mins [RR] [SS] isomers 32mins	0.01 mg kg^{-1}	88-104% (0.01-1.2 mg kg^{-1})	Reference 103
					or 1.8m 4% SE30 6% QF1 at 225°C.	[RS] [SR] isomers 24mins [RR] [SS] isomers 26mins			
Cabbage	hexane: acetone	-	Florisil	benzene: ethyl acetate	electron capture 0.5m 3% OV101 +3 Apiezon L 225°C.	no separation of [RR], [SS], [RS], [SR]	0.01 mg kg^{-1}	not given	Reference 105

deltamethrin residue using procedures similar to those of Mestres et al (Ref. 102). The residue is then transesterified by treatment with methanolic potassium hydroxide and the resulting methyl ester of 3-(2,2-dibromovinyl)-2,2-cyclopropanecarboxylic acid is quantitatively determined by GC using a non-polar stationary phase and electron capture detection. This method was reported to be very sensitive, with levels of deltamethrin of 0.0035 mg kg^{-1} being measured.

5.5 Fenpropathrin

The publications by Chapman et al (Refs. 91 and 92) refer to the determination of residues of fenpropathrin as modifications of the permethrin methodology described in Table 1. the insecticide was chromatographed using OV-101 with an electron capture detector, the linear range being reported as 10-100 pg injected. Naturally occurring components in crop extracts were reported to interfere with fenpropathrin determination but these could be selectively removed by Florisil or aluminium oxide cleanup using benzene:hexane elution from the adsorption columns.

5.6 Fenvalerate

The published literature contains four references to fenvalerate residue analytical methodology. All methods are based upon the determination of the intact ester by gas liquid chromatography and two are adaptations of procedures described for permethrin analysis, modifying only the final GLC conditions to accommodate fenvalerate. The methodology used by Lee et al (Ref. 104) and Talekar (Ref. 105) for fenvalerate residue determinations are outlined in Table 3. Lee (Ref. 104) reported that naturally occurring compounds from cabbages coextracted with fenvalerate and eluted at very long GC retention times. Use of an additional silica gel cleanup procedure removed this component however and considerably shortened the effective analysis time. The choice of percentage loading and stationary phase for GC of fenvalerate would appear to be very important. Talekar reported that the use of 3% SE-30 gave five peaks, two with short retention times (<4 mins), and three with long retention times (~60 mins), for fenvalerate and that 10% DC-200, 2% OV-1, 2% OV-1+3% QF-1 (1.8m) were unsuitable for fenvalerate analysis. However, 5% OV-101 and 10% QF-1 columns were successfully used by Chapman and Simmons whilst 15% OV-101 or 4% SE-30+60% QF-1 were reported to be satisfactory by Lee. It should be noted that Talekar used stainless steel GC columns and this may account for the variable and sometimes contradictory results obtained.

5.7 Degradation Products of Pyrethroids

No publications were found in the open literature describing the residue analytical determination of breakdown products of the synthetic pyrethroids. However previously described methodology (Ref.s 81,86,98 & 103) for the products of hydrolysis of pyrethroids could be modified to analyse for these compounds when they occur as natural breakdown products. Lam and Grushka (Ref. 106) reported an HPLC separation of permethrin and its metabolites, 3-phenoxybenzyl alcohol and 3-phenoxybenzoic acid, but no indication of sensitivity or residue methodology was given.

6 CONCLUSIONS AND RECOMMENDATIONS

The pyrethroids discussed in this review have been extensively and thoroughly studied over the past few years. More than 100 references appear in the published literature and, as a result of these, the residue chemistry and environmental behaviour is, in general, well understood. In addition, a considerable number of studies have been carried out but not yet published. Encouragement should be given to the publication of much of this additional information so that a more complete story appears in the open literature.

On the basis of the available publications, there is adequate information, in most cases, to make a clear judgement on the environmental impact of the pyrethroids reviewed here.

In a few areas, mainly from the standpoint of academic interest, some additional points could be clarified in more detail for completeness.

6.1 Photochemistry

It has been clearly indicated that light has significant effects on the degradation of pyrethroids. The nature of the reaction and the rate depend on the chemical structure, the light source, the surrounding condition as well as the presence of sensitiser and quencher. The main photo-reactions occurring in the pyrethroids can be summarised as follows (see also Ref. 107).

(a) R/S epimerisation;
(b) trans/cis isomerisation;
(c) reductive dehalogenation including those encountered with halovinyl substituents;
(d) photoelimination of carbon dioxide
(e) hydrolytic cleavage of ester bond and ether bond;
(f) oxidation of the parent pyrethroids and the cleavage products;
(g) dimerisation of free radicals.

Several pyrethroids have been extensively studied with respect to photolysis. However, further photodecomposition studies should be encouraged based on the following considerations.

(i) The present knowledge on pyrethroid photolysis has been mainly acquired with selected compounds like permethrin, deltamethrin and fenvalerate, and no detailed information is available on phenothrin, cypermethrin and fenpropathrin. However, photolysis of several synthetic chrysanthemates has been well pursued, and the findings will be relevant for elucidating the decomposition of certain pyrethroids.

(ii) Not all photoproducts and photoreaction pathways have been elucidation as yet, and a substantial portion of the applied radioactivity is still unidentified in some cases.

(iii) So far, most of the photolysis studies have been carried out under somewhat artificial conditions (e.g. in organic solvents and using aritificial light). Many photochemical products have been identified and the information has been useful in providing insight into the photochemistry of pyrethroids. However, what is required, in addition, is information relevant to the practical usage of the compounds. In the aqueous environment, pyrethroids are rapidly removed from the aqueous phase by adsorption on to sediments. Studies of photolysis in water/sediment systems and on the soil surface under natural sunlight and therefore highly relevant.

6.2 Metabolism in Plants

(a) There is a considerable amount of information on the metabolism of the pyrethroidal compounds except (fenpropathrin) on cotton, bean foliage and other plants. Generally very little translocation of pyrethroids to the untreated area occurs. Experiments in which plants were grown in soil treated with [^{14}C]-permethrin or [^{14}C]-fenvalerate showed that some uptake of degradation products occurred. However, this process is unlikely to give rise to significant amounts of residues in field situations. Based on the available information, the terminal residues of the pyrethroids in plants are likely to be mainly unchanged parent compounds, with possibly some free and conjugated metabolites derived from the acid and alcohol moieties.

(b) The trans isomers of permethrin, cypermethrin and deltamethrin were hydrolised more rapidly than the cis isomers, and oxidation occurred to a greater extent with the more stable cis isomer. Some interconversion of cis to trans isomers was observed. With permethrin, a portion of the trans isomer was converted to cis permethrin. Several oxidised ester metabolites were detected with all the pyrethroids studied.

Phenothrin is the least photochemically stable of the pyrethroids discussed with a half-life of less than one day, and rapid degradation of the photochemical products also occurred.

(c) A large number of metabolites were formed mainly as a result of hydrolysis at the ester bond and/or hydroxylation of the acid and alcohol moieties. Conjugation of the various carboxylic acids and hydroxylated products occurred and some products formed more than one type of conjugate. The major metabolites were PBalc and PBacid, and acid moieties such as Cl_2CA, Br_2CA and ClVacid, which occurred also as conjugates.

Those pyrethroids containing the α-cyano moiety, namely cypermethrin, deltamethrin and fenvalerate produced more hydrolysis products from the alcohol moiety than did permethrin, and some evidence of the formation of small amounts of PBcy has also been obtained. In addition, the amide analogs of cypermethrin and fenvalerate were formed.

The plant conjugates derived from fenvalerate and cypermethrin have been fairly extensively investigated.

Thus, although the plant metabolism of these pyrethroids have been extensively studied, further studies should be encouraged:

(i) The available data suggest that the products found on plants are similar in glasshouse and outdoor experiments. However, only few outdoor experiments have been carried out and additional information would be interesting. Although some photodegradation products are the same as "metabolites" (e.g. ester cleavage products) the possibility that other photochemical conversion products could appear as terminal residues in plants should not be overlooked.

(ii) In use, pyrethroids are applied to a wide range of crops and additional metabolism data on other crops to confirm similar metabolic pathways would be useful. These studies should include crops grown to maturity.

6.3 Metabolism in Soil and by Soil Microorganisms

There is ample basic information about the degradation of permethrin, cypermethrin, phenothrin, fenpropathrin and fenvalerate in soils. Where isomers exist, the trans-isomer is degraded more rapidly than the cis-isomer. All compounds are degraded primarily by hydrolysis at the ester bond and there is evidence for further degradation of cleavage products. However, in some soils and under anaerobic conditions, some hydrolysis products have been shown to degrade slowly. In addition, under aerobic conditions, hydrolysis at α-cyano groups occurs, where appropriate, and, in the case of fenvalerate, ether cleavage has been reported.

There appears to be little likelihood of pyrethroids or their metabolites persisting for lengthy periods in soil. However, attention needs to be paid to the possible uptake by plants of soil metabolites that are not plant metabolites.

It has been shown that Cl_2CA and a cyclopropane dicarboxylic acid arising from permethrin degradation in soil could be taken up by plants in a model experiment, but residues of these compounds are unlikely to result in crops following field use of permethrin.

6.4 Metabolism in Animals

The in vivo metabolism of the recently developed synthetic pyrethroids has been extensively studied mainly in rats but also in some other species. The results can be summarised as follows.

(a) Orally administered pyrethroids are rapidly metabolised, and eliminated in urine and faeces, and radioactive residues, except the cyano fragment do not persist in the animal body.

(b) In the faeces, some portions of the administered compound are found unchanged. This is probably due to rather slow absorption from the gastrointestinal tract rather than to entero-hepatic circulation, since there is no evidence for intact fenpropathrin in the bile (Ref. 71).

(c) The excreted metabolites have been well identified, especially with permethrin, deltamethrin and fenvalerate. Although several ester metabolites are excreted in the faeces, the major portion of the administered dose is subject to hydrolysis at the ester linkage before or after oxidation at various sites (depending on the structure) of both alcohol and acid moieties. In the alcoholic moiety, the 4'-position of the phenoxy ring is the sole or major site for hydroxylation, and, in the acid moiety, the alkyl groups are oxidised.

These primary metabolic products are conjugated to varying degrees with glucuronic acid, sulphate, glycine, glutamic acid and/or taurine.

(d) The rate of hydrolysis and/or oxidation depends on the chemical structure including configuration of the molecule. For example, cis-permethrin and cis-phenothrin are more slowly hydrolysed, and as a result more easily oxidised, than the trans counterparts.

On the basis of the review of published data there are still aspects of the animal metabolism where further information is needed.

(i) Some portions of the administered radioactivity still remain to be identified either in the tissues or excreta even of rats. Work on their identification is desirable for the complete understanding of the metabolic fate of pyrethroids.

(ii) Most of the studies so far involved a single oral administration. Further work is desirable using repeated doses at a relatively low level since the metabolic pattern may be different under such conditions. Such studies would give useful information regarding possible build up of residues in animals.

(iii) The literature contains information on the metabolism in domestic animals (e.g. ruminants and poultry) for only one pyrethroid, permethrin. Information is needed for some of the other pyrethroids but is undoubtedly available although currently unpublished.

(iv) Information is needed on the residues of metabolites that might be present on the edible portion of crops treated under practical conditions.

(a) If residues of metabolites occur in such crops that are used as animal food then the fate of these metabolites in domestic animals will need to be considered.

(b) If residues of metabolites occur in such crops that are different from the mammalian metabolites, information on their fate might be needed in species, such as rats and mice.

6.5 Residue Analysis Procedure

(a) Currently developed synthetic pyrethroids for agricultural use are very similar in terms of structure, polarity and lipophilicity. It should, therefore, be expected that very similar residue analytical methods would be developed for individual compounds and this is indeed the case for procedures reviewed here.

(b) In general, analytical methodology leading to the final determination of low residues of the intact esters, in good recovery, have been reported. All the individual pyrethroids can be measured by modification of the final determination step, except phenothrin which has very low sensitivity to the detectors. Hence, this should allow the development of straightforward multiresidue procedures.

(c) Gas chromatography-mass spectrometry is an ideal instrument for the qualitative and quantitative confirmation of residues, particularly as many of synthetic pyrethroids esters share common acid or alcohol moieties which give rise to common ions in their mass spectra.

(d) Additional alternative/confirmation techniques are available involving the initial hydrolysis and subsequent derivatisation of the acid/alcohol moieties. These methods are necessarily more time-consuming and prone to error than the direct ester determination methods and it is therefore doubtful if they are suitable for routine use.

(e) However, transesterification using optically pure alcohols provides both a confirmation technique and the facility of separating the optical isomers of pyrethroid residues.

Based on the existing information on residue analytical procedures of the pyrethroids, the following research needs should be suggested.

(i) As methodology for the determination of the metabolic breakdown products of the synthetic pyrethroids is not yet available in the literature, analytical procedures should be developed.

(ii) Validation of the methodology which has appeared in the literature to date is unfortunately lacking. Particularly significant is the lack of extractability studies using "weathered" residues and an investigation of the potential for interference of the procedures arising from ubiquitous contaminants such as phthalate esters, polychlorinated biphenyls, and, indeed, organochlorine insecticides.

(iii) Similarly very little rationalisation of the methodology used in order to determine the "optimum" conditions for individual procedural steps has been reported.

(iv) Many workers continue to advocate the use of solvents, e.g. benzene, which are virtually unacceptable for the general use in modern, safety conscious laboratories. Alternative procedures should be further worked out.

6.6 General Recommendation

It is probable that much of the additional work suggested above in 6.1 to 6.5 has already been done and if so publication of the results should be encouraged.

In this review, information on the behaviour of the pyrethroids in the aquatic environment has been ommitted since only little published information is available yet. (Ref. 108 and 109). However, information concerning accumulation and/or metabolism in aquatic organisms is needed under circumstances where river, pond and sea water could be contaminated with residues of pyrethroids and their degradation products.

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