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Permethrin Metabolism in Rats and Cows and in Bean and Cotton Plants

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The discovery that 3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (permethrin) combines outstanding insecticidal activity, low mammalian toxicity and adequate stability in light and air has focused attention on the potential of synthetic pyrethroids in agricultural pest insect control (1,2). Most permethrin preparations are [1RS, trans,cis]-mixtures, the [1R,trans]- and [1R,cis]-isomers being the insecticidal components (3). The importance of understanding permethrin biodegradation prompted the present study on the comparative metabolism of [1R,trans]-, [1RS, trans]-, [1R,cis]- and [1RS,cis]-permethrin in rats (4,5) and of [1RS,trans]- and [1RS,cis]-permethrin in cows and in bean and cotton plants.

[^{14}C]Permethrin Preparations and Experimental Procedures for Rats and Cows

Eight [^{14}C]permethrin preparations were used with specific activities ranging from 1.7 to 58.2 mCi/mmole (Figure 1). The [1R]-isomers were prepared as previously reported (4) and the [1RS]-isomers were provided by FMC Corporation (Middleport, N.Y.). Rats (male, albino, Sprague-Dawley strain) treated with a

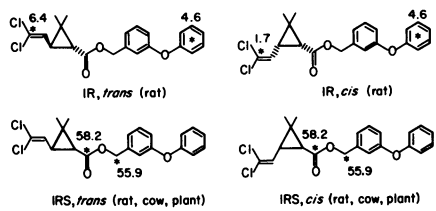

Figure 1. Eight preparations of ^{14}C-permethrin (specific activity, mCi/mmol)

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single oral dose of each of the 8 labeled preparations at 1-4 mg/kg were held 4- or 14-days in metabolism cages and then sacrificed for radioanalysis of urine, feces, CO_2 and tissues. Metabolites in the urine (40-100 μl) and in the feces (methanol extract equivalent to 40-230 mg feces) were subjected to tlc cochromatography on silica gel chromatoplates with standard compounds (6) or their methylated (CH_2N_2) derivatives or with glucuronides synthesized enzymatically (5). Individual metabolites isolated by tlc were also hydrolyzed with enzymes (β-glucuronidase, aryl sulfatase) or with acid and base to obtain cleavage products for tlc cochromatographic identification with and without derivatization. Urinary metabolites were chromatographed in acidic solvent systems to move all products free from the origin. The fecal metabolites were chromatographed in both acidic and neutral solvent systems, the latter to minimize decomposition of ester metabolites.

The studies comparing all 8 [^{14}C]preparations in rats gave very similar results for the $Cl_2C^*=$ and -$C^*(O)$- labels in the acid moiety and for the -C^*H_2- and phenoxy* labels in the alcohol moiety and with no $^{14}CO_2$ production in any case. These findings indicate that either of the [^{14}C]acid preparations or [^{14}C]alcohol preparations can be used to detect all of the metabolites from the acid and alcohol moieties, respectively. In addition, the [1R]- and [1RS]-isomers gave almost identical results, so the [1RS]-isomers with specific activities of 55.9-58.2 mCi/mmole were used in the studies with cows and other organisms.

Cows (lactating Jersey, arrangements by FMC Corporation) treated with 3 consecutive daily doses by intubation into the rumen of the 4 labeled preparations of [1RS]-permethrin at 1 mg/kg were held 12- or 14-days prior to sacrifice and analyses as above.

[^{14}C]Permethrin Metabolites in Rats and Cows

The [1RS,*trans*]-isomer of permethrin yields more urinary radiocarbon than [1RS,*cis*]-permethrin with either acid- or alcohol-labeled preparations and with either rats or cows (Table I).

Table I. Percent Urinary Radiocarbon from [1RS,*trans*]- and [1RS,*cis*]- [^{14}C]Permethrin Preparations

Isomer and label position	Rats	Cows
1RS,*trans*		
Acid	82	39
Alcohol	79	47
1RS,*cis*		
Acid	54	29
Alcohol	52	22

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The majority of the metabolites appear in the urine with rats and in the feces with cows. These results indicate more extensive ester cleavage or conjugation of the metabolites with trans-permethrin than with cis-permethrin and in rats as compared to cows.

The tlc cochromatographic technique for metabolite identification is illustrated in Figure 2 with the metabolites from the acid moiety of [1RS,trans]-permethrin in rats and cows.

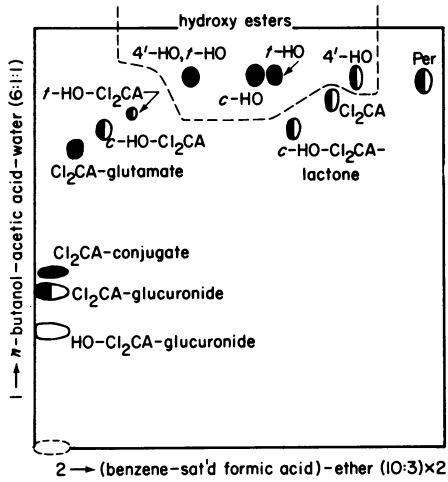


Figure 2. Metabolites from acid moiety of (1RS, trans*)-permethrin. ○ represents rat; ●, cow; and ◑, rat and cow.*

The solvent system for the first direction of development separates the conjugates and the second solvent system then resolves permethrin, its derivatives with monohydroxylation at the trans- or cis-position of the gem-dimethyl group relative to the carboxyl group (t-HO; c-HO), its 4'-HO derivative from phenoxy hydroxylation, its dihydroxy derivative (4'-HO,t-HO), the acid moiety (Cl_2CA) and its hydroxy derivatives (t-HO-Cl_2CA; c-HO-Cl_2CA), and the lactone of c-HO-Cl_2CA (from cyclization before excretion or as an artifact from cyclization on analysis). Most of the metabolites are formed by both rats and cows. However, only cows give ester metabolites hydroxylated at the gem-dimethyl group, the glutamate conjugate of Cl_2CA and an additional unidentified metabolite of the acid moiety. In contrast, only rats form glucuronides of the HO-Cl_2CA derivatives.

Studies of the type indicated above with each labeled preparation of [1RS,trans]- and [1RS,cis]-permethrin served to define the sites of metabolic attack in rats and cows (Figure 3). There are 4 principal sites of attack in each case, with an additional site for rats administered [1RS,cis]-

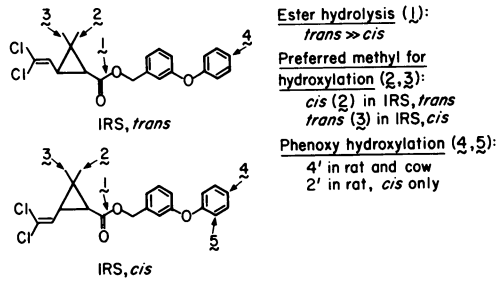


Figure 3. Sites of metabolic attack from rat and cow

permethrin. Ester hydrolysis (1) is more rapid with trans- than with cis-permethrin. Oxidation at the gem-dimethyl group occurs selectively at the cis-position (2) in [1RS,trans]-permethrin and at the trans-position (3) in [1RS,cis]-permethrin. The phenoxy group is hydroxylated at the 4'-position (4) with both isomers in rats and cows and at the 2'-position (5) with cis-permethrin in rats only.

Eight ester metabolites hydroxylated in the acid or alcohol moiety are identified from the feces of rats and cows (Figure 4). Three of the 4 possible esters from monohydroxylation at the gem-dimethyl group appear in cow feces but only the 2-trans-hydroxy compound from the more metabolically-stable cis-permethrin isomer appears in rat feces. The 4'-hydroxy derivative is present with both trans- and cis-

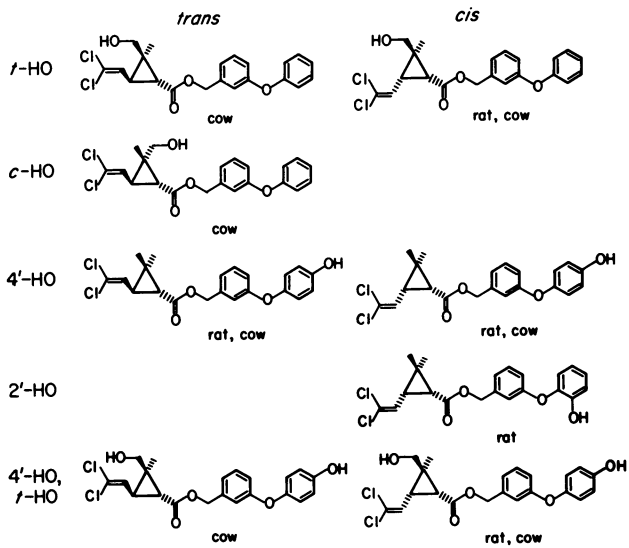
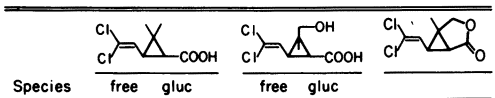


Figure 4. Rat and cow hydroxy ester metabolites

permethrin and in both rats and cows. The 4'-HO,t-HO-derivative appears only in cows with trans-permethrin and in both rats and cows with cis-permethrin. The feces of rats administered cis-permethrin contains the 2'-hydroxy derivative. These species differences are attributable in part to less extensive ester hydrolysis in cows than in rats and the ability of rats to carry out aryl hydroxylation at the 2'-position.

The acid moieties from [1RS]-trans- and -cis-permethrin are mostly excreted in rats and cows as the corresponding glucuronides. The other metabolites are also the same in both species except that in cows the glucuronides of the hydroxy acids are not detected and Cl_2CA is conjugated in part with glutamic acid (Figure 5).

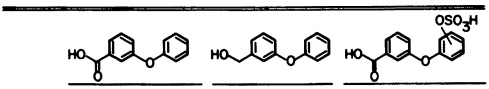


Species	free	gluc	free	gluc	
Rat	+	++	+	+	+
Cow	+	++*	+	-	+

*also glutamate conjugate of *trans*-acid

Figure 5. Metabolites from acid moiety of (1RS, trans)-permethrin and (1RS,trans)-permethrin, rat and cow

The alcohol moiety liberated on cleavage of [1RS,trans]- and [1RS,cis]-permethrin is in the most part further oxidized to the corresponding benzoic acid which is excreted free in rats, as a glycine conjugate and glucuronide in rats and cows and as the glutamate conjugate which is the major metabolite in cows but absent in rats (Figure 6). 3-Phenoxybenzyl



Species	free	gly	gluc	glut	free	gluc	4'	2'
Rat	+	+	+	-	+	-	++	+
Cow	-	+	+	++	+	+	+	-

Figure 6. Metabolites from the alcohol moiety of (1RS, trans)-permethrin and (1RS, cis)-permethrin, rat and cow

alcohol is excreted free in rats and cows and as a trace amount of glucuronide in cows only. The major rat metabolite, the sulfate of the 4'-hydroxy acid, is present in small amount in cow urine and the sulfate of the 2'-hydroxy derivative appears only in rat urine.

The complete metabolic pathway for trans- and cis-permethrin in rats including the 24 identified metabolites (5) is shown in Figure 7. This pathway accounts for all permethrin metabolites excreted in amounts of >1% of the administered radiocarbon except for 5 minor fecal metabolites of cis-permethrin.

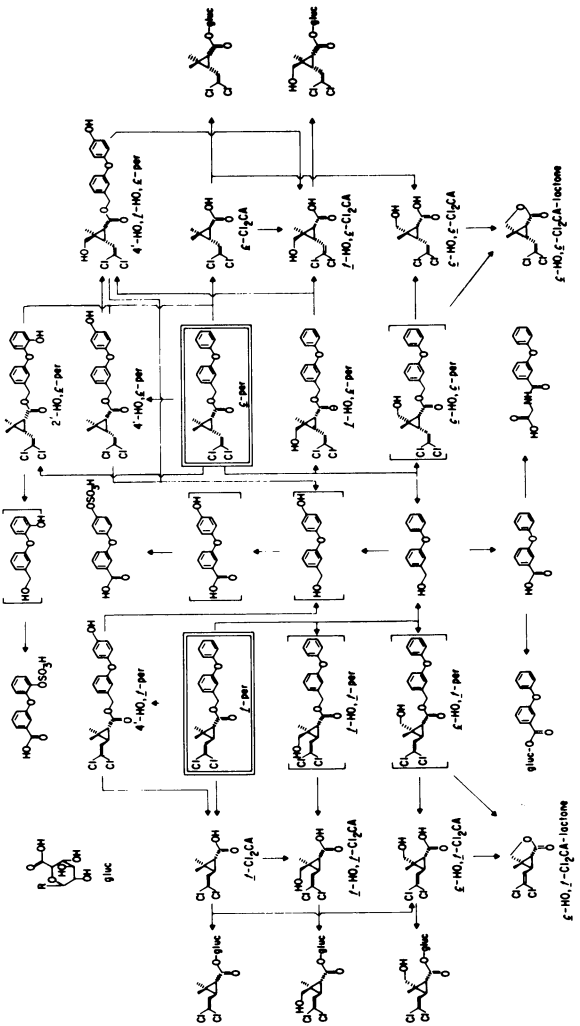


Figure 7. The complete metabolic pathway for trans- *and* cis-*permethrin*

Experimental Procedures for Bean and Cotton Plants and [^{14}C]-Permethrin Metabolites in Plants

Bean and cotton plants treated topically on the leaves and bean plants treated by stem injection with the 4 [^{14}C]-preparations of [1RS]-permethrin were held for up to 21 days in the greenhouse. The leaves were washed with methanol-chloroform (2:1) and then extracted with this solvent mixture or the whole plants were extracted in the same manner. Plant metabolites were identified and analyzed by the methods used for the mammalian metabolites, except that β-glucosidase, cellulase and acid were employed for conjugate cleavage.

An extract of bean plants 14 days after stem injection with [1RS,*trans*]-permethrin contains the parent compound, hydroxylated permethrin, the free dichlorovinyl acid and its hydroxy derivatives, phenoxybenzyl alcohol and phenoxybenzoic acid. These products appear as one spot in the *n*-butanol-acetic acid-water solvent system (Figure 8) but they are resolved in the benzene(formic acid)-ether system. The products at Rf 0.47 and 0.61 are conjugates of hydroxylated permethrin while the Rf 0.56 product is an unidentified conjugate from the acid moiety. The identified conjugates include the glycosides of the dichlorovinyl acid and of 3-phenoxybenzyl alcohol.

Permethrin on bean and cotton leaves undergoes *trans-cis* isomerization to the extent of 6-13% in 21 days. The penetrated portion yields metabolites similar to those found in the injected bean plants. In all cases, *trans*-permethrin is more rapidly metabolized than *cis*-permethrin.

These preliminary results with plants indicate the importance of photodecomposition and metabolic oxidation and hydrolysis in the dissipation of permethrin residues.

Abstract

Permethrin metabolites excreted by rats and cows include 8 mono- and dihydroxy derivatives of the *trans*- and *cis*-esters, the acid moieties from ester cleavage and their 2-*trans*- and 2-*cis*-hydroxy derivatives, 3-phenoxybenzyl alcohol, and 3-phenoxybenzoic acid and its 2'- and 4'-hydroxy derivatives. These metabolites are excreted without conjugation or as glucuronides and glycine and glutamic acid conjugates of the carboxylic acids and as sulfates of the phenolic compounds. Permethrin on bean and cotton leaves undergoes *trans-cis*-photoisomerization and the absorbed material yields hydroxy esters and their glycosides, hydrolysis products and their glycosides, and 3-phenoxybenzoic acid. *trans*-Permethrin generally undergoes more rapid biodegradation than *cis*-permethrin, in part because of the greater hydrolysis rate of the *trans*-compound.

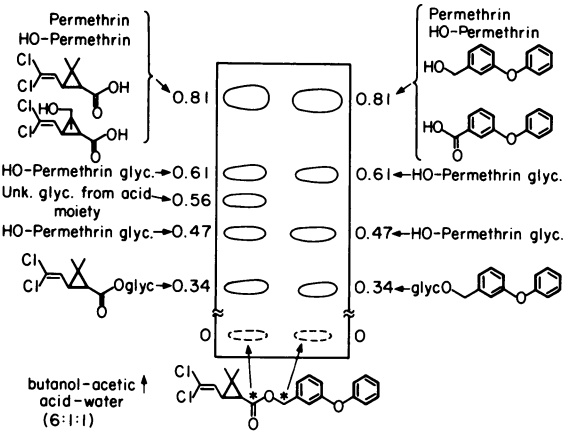


Figure 8. Bean metabolites of (1RS, trans)-permethrin, stem injection

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