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Pyrethroids: Mammalian Metabolism and Toxicity†

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ABSTRACT: Synthetic pyrethroids, a major insecticide group, are used worldwide to control agricultural and household pests. Mammalian metabolism of pyrethroids was substantially launched in the 1960s and 1970s by the research groups of Professor Casida and Sumitomo Chemical Co., which made great contributions to the elucidation of their metabolic fates. They showed that ester hydrolysis and oxidation play predominant roles in mammalian metabolism of pyrethroids and that rapid metabolism leads to low mammalian toxicity. These metabolic reactions are mediated by carboxylesterases and CYP isoforms, the resultant metabolites then undergoing various conjugation reactions. In general, there are substantially neither significant species differences in metabolic reactions of pyrethoids nor metabolic differences among their chiral isomers except with fenvalerate, one isomer of which yields a lipophilic conjugate causing toxicity.

KEYWORDS: pyrethroids, mammalian metabolism, toxicity, fenvalerate

INTRODUCTION

First, I would like to cordially express my respect to Professor J. E. Casida for great achievements in pyrethroid research. In addition, I offer my thanks to the organizers of this symposium for inviting me. It is a great honor to have an opportunity to make a presentation about pyrethroid metabolism and toxicity on this occasion.

Natural pyrethrins have been used for the control of mosquitoes since ancient times, and many synthetic pyrethroids have been developed by modification of their chemical structures for better biological performance and stability in the environment.[1] The primary target site of toxic actions of pyrethroids in mammals is reported to be voltage-sensitive sodium channels. In addition, voltage-gated calcium channels, voltage-gated chloride channels, and $GABA_A$ receptors may contribute to the neurotoxic effects of at least some pyrethroids.[2]

Synthetic pyrethroids can be classified into two categories: first and second generation.[1] The characteristic feature of the first-generation pyrethroids, which are esters of chrysanthemic acid derivatives and alcohols having a furan ring and terminal side-chain moieties, is sensitivity to light, air, and temperature. Therefore, these pyrethroids have been used mainly for the control of indoor pests. On the other hand, the second-generation pyrethroids, generally 3-phenoxybenzyl alcohol derivatives, have excellent insecticidal activity as well as sufficient stability in the environment. Thus, second-generation pyrethroids have been used worldwide for the control of agricultural pests.[1]

Pyrethroids constitute one major insecticide group commercially, and a 2008 survey[3] indicated that the global market value for insecticides was about $11000 million, with pyrethroids accounting for 18%, following neonicotinoides (21%) and organophosphates (20%) by narrow margins. More than 20 pyrethroids have been marketed for agricultural pest control, and the leading ones in 2008 were λ-cyhalothrin, deltamethrin, cypermethrin, bifenthrin, and α-cypermethrin in terms of total sales.[3]

† Part of the Symposium on Pesticide Toxicology in Honor of Professor John Casida. The following researchers from Sumitomo Chemical Co., Japan, were involved in pyrethriod metabolism studies as postdoctoral researchers in J. E. Casida's laboratory: Dr. Y. Nishizawa (1960s) and Dr. K. Ueda (1970s).

From the historical viewpoint, mammalian metabolism studies of pyrethroids can be roughly divided into three periods (first period, from the late 1960s to the mid-1970s; second period, from the mid-1970s to 2000; third period, 2000 to the present). During the first period, mammalian metabolism studies of the first-generation pyrethroids was substantially launched by the research groups of Professor Casida and Sumitomo Chemical Co., and these groups showed their metabolic reactions and identification of metabolites mainly in rodents. In 1973, the book *PYRETHRUM, The Natural Insecticide* (Academic Press, New York and London) edited by Professor Casida was published, which is the first, to my knowledge, to deal with metabolism and toxicology of pyrethroids.

In the second period, many in vivo and in vitro metabolism studies of the first- and second-generation pyrethroids were carried out, and metabolic fates were extensively examined in several mammalian species including humans, mostly using radiolabeled preparations. Furthermore, geometrical and chiral isomers of pyrethroids were the focus of attention for better understanding of metabolic pathways. Since 2000 (third period), molecular biology has made great progress, and accordingly genetically expressed CYP isoforms or carboxylesterases of animals or humans have become available. Furthermore, human hepatic microsomes or frozen hepatic cells have been on the market. Therefore, it has become possible to determine which enzymes are responsible for metabolic reactions and to detect clear species differences between humans and laboratory animals.[1]

OVERVIEW OF MAMMALIAN METABOLISM

So far, metabolic studies of about 30 synthetic pyrethroids, including their chiral and geometrical isomers, have been carried out in mammals and reviewed.[1] However, detailed metabolism data have not been necessarily published in scientific journals. In

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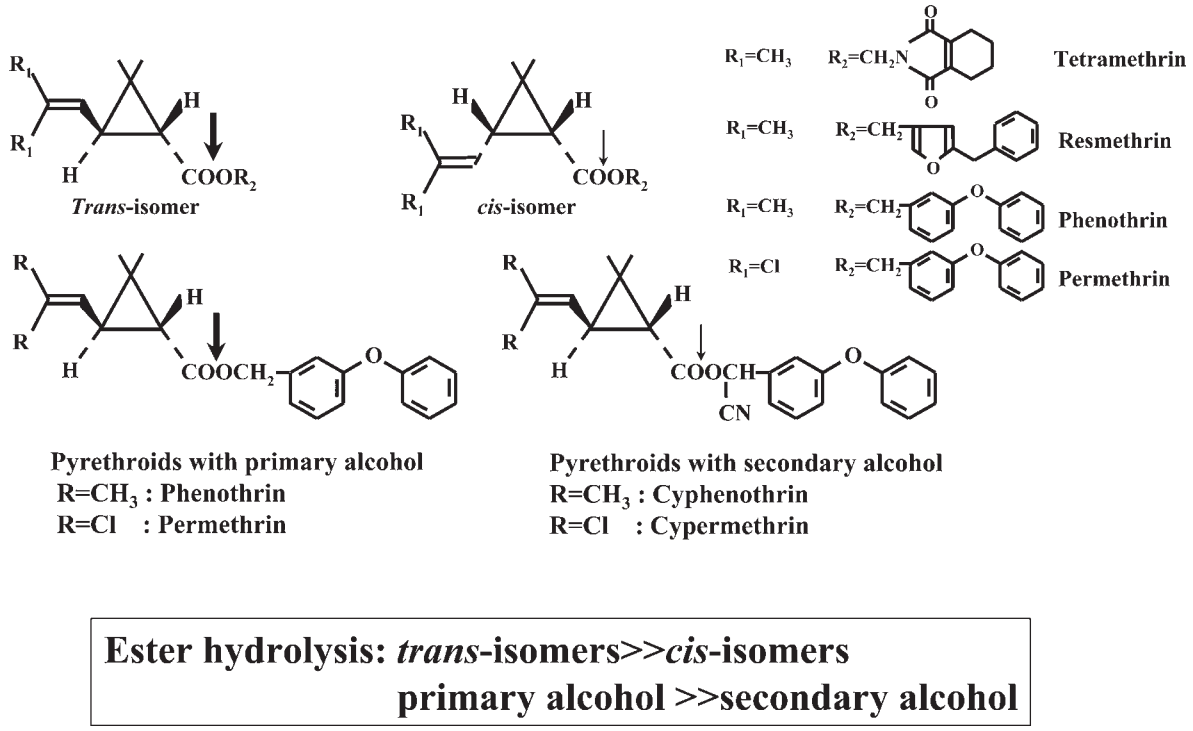

Figure 1. Metabolic reaction 1: ester hydrolysis.

some cases, the reports of joint World Health Organization/Food and Agricultural Organization (WHO/FAO) expert meetings on pesticide residues and the International Programme on Chemical Safety (IPCS), Environmental Health Criteria (WHO), were referred to ref 1.

Absorption, Tissue Distribution, and Excretion. With regard to absorption, in general, oral absorption rates are rather high in rats and mice, and dermal penetration rates are low. However, oral absorption rates depend on the vehicles used for dosing. After systemic absorption, pyrethroids and their metabolites do not show accumulation in any specific tissues or organs.[1] The acid and alcohol moieties of pyrethroids are rapidly and completely excreted into urine and feces within several days after oral administration.[1] However, the carbon derived from the CN group of pyrethroids, which are α-cyano-3-phenoxybenzyl alcohol derivatives, shows incomplete excretion and longer bioretention in skin and stomach.[4−8] This slow and incomplete excretion is likely due to distribution to the extracellular fluid and partial binding with serum albumin, as is the case with endogenous thiocyanate.[4,5]

Metabolic Pathways. A review of the metabolic pathways of about 30 pyrethroids revealed that the major metabolic reactions are commonly oxidation, ester hydrolysis, and conjugation in all cases.[1] These metabolic reactions proceed in animals in first and second steps. As a first step, so-called phase I reactions occur, which are oxidation and ester hydrolysis. The second-step conjugation is a phase II reaction to generate hydrophilic and lipophilic forms. Hydrophilic conjugates are often found as glucuronides, sulfates, or amino acid conjugates, and these are readily excreted into urine due to high water solubility. In less frequent cases, lipophilic conjugates are found, these generally showing longer bioretention than their hydrophilic ones. Although data in the public domain are limited, metabolites of pyrethroids normally show less acute oral toxicity than parent compounds so that rapid metabolism leads to low mammalian toxicity.[9]

Metabolic Reactions. *Phase I Reactions.* Ester hydrolysis occurs to a larger extent with the *trans* and primary alcohol derivatives as compared with the corresponding *cis* and secondary alcohol derivatives, respectively. The chirality (1*S* or 1*R*) at the acid moiety of phenothrin,[10] tetramethrin,[11] and permethrin[12] does not significantly affect ester hydrolysis (Figure 1). Oxidation reactions occur on several sites of the acid and alcohol moieties, depending on the chemical structure. For example, the *trans* methyl of the isobutenyl group in chrysanthemates is preferentially oxidized over the *cis* methyl group, and the 4′-position of the phenoxy ring is oxidized to a larger extent as compared with other positions (Figure 2).[13]

Phase 2 Reactions. Hydrophilic conjugates found in mammalian metabolism of pyrethroids are glucuronides, sulfates, and amino acid conjugates (Figure 3).[1] 3-Phenoxybenzoic acid (3-PBacid), a common metabolite from pyrethroids having a 3-phenoxybenzyl alcohol or α-cyano-3-phenoxybenzyl alcohol in the alcohol moiety, shows remarkably diversified amino acid conjugates: a glycine conjugate is the major form in sheep, cats, and gerbils; a taurine conjugate is found in mice; and a glycylvaline dipeptide conjugate is found in the mallard duck.[14] In addition, thiocyanate and sulfonic acid conjugates have been reported. Thiocyanate is formed by conversion of the CN ion released from ester hydrolysis of pyrethroids with the α-cyano-3-phenoxybenzyl alcohol derivative.[4−8] Sulfonic acid conjugates have a sulfonic acid group incorporated into the double bond of the 3,4,5,6-tetrahydrophthalimide moiety of tetramethrin and are reported to be formed in the intestinal tract by nonenzymatically direct addition of sulfonic acid.[15] A mercapturic acid conjugate is documented to be involved in the metabolism of prallethrin (Figure 3).[16]

In addition, three types of lipophilic conjugates have been reported from pyrethroid metabolism studies. These are cholesterol ester (fenvalerate),[17,18] glyceride (a metabolite: 3-PBacid),[19] and bile acid conjugates (fluvalinate) (Figure 4).[20]

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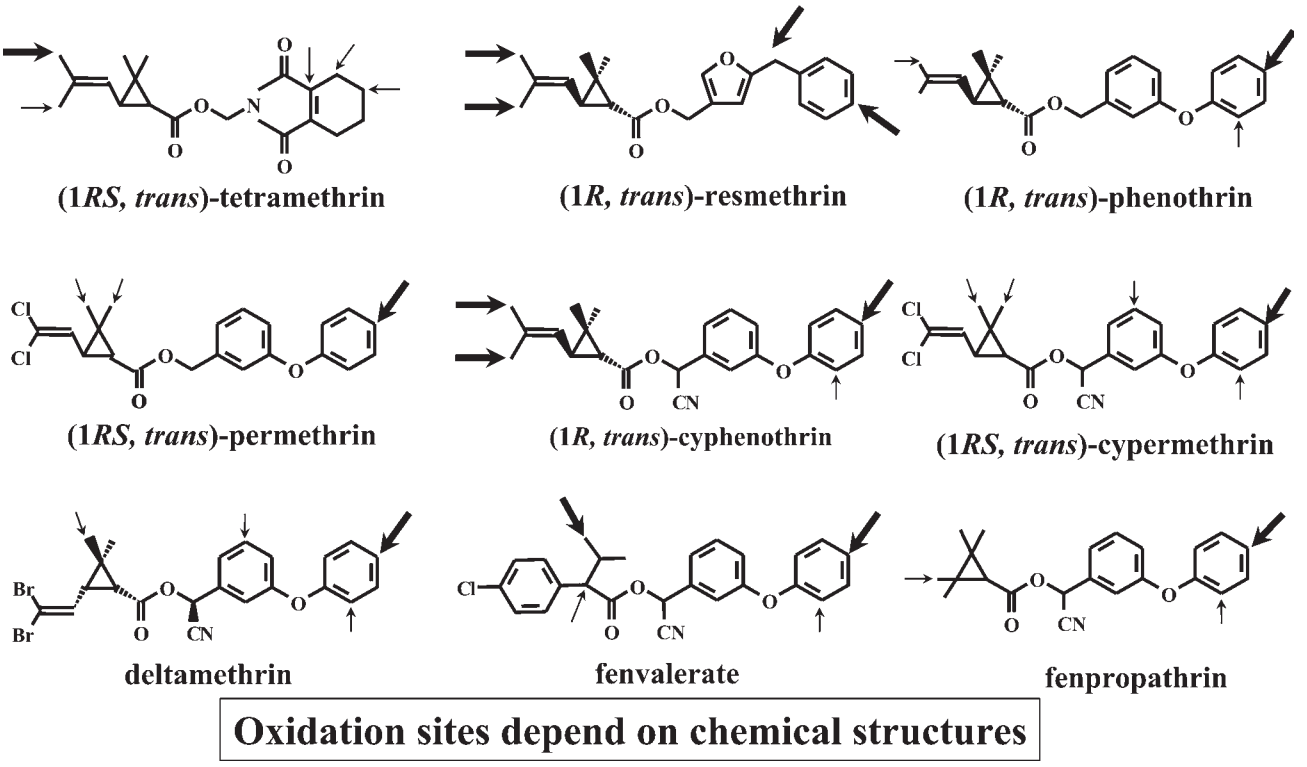

Figure 2. Metabolic reaction 2: oxidation.

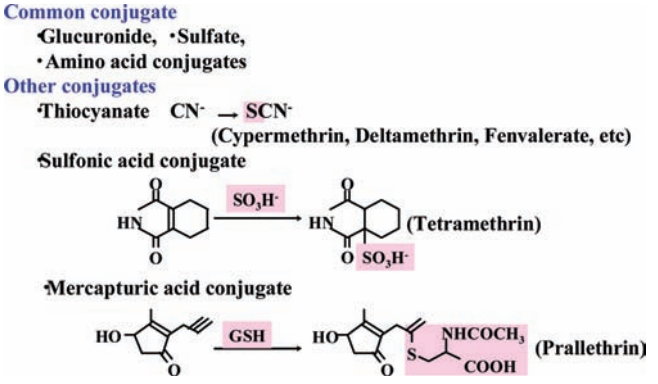

Figure 3. Metabolic reaction 3: hydrophilic conjugates.

METABOLIC DIFFERENCES AMONG OPTICAL ISOMERS OF PYRETHROIDS

In general, pyrethroids have chiral isomers due to the presence of chiral centers. Whereas no substantial differences have been described for absorption, excretion, or metabolic reactions among chiral isomers of phenothrin,[10] tetramethrin,[11] and permethrin,[12] fenvalerate shows a significant difference in formation of a lipophilic conjugate. It has four chiral isomers due to the presence of two chiral carbons, and one of these, the Bα (2*R*, α*S*)-isomer, yields a cholesterol ester conjugate from its acid moiety (Figure 5).[17] This conjugate is demonstrated to be a causative agent for granulomatous changes, which are observed in rats and mice when fenvalerate is dosed for a long time.[21] In addition, this chiral-specific formation of the cholesterol ester has been demonstrated to be mediated by transesterification reactions of carboxylesterase(s) in microsomes, but not by any of the three known biosynthetic pathways of endogenous cholesterol esters (acyl-CoA:cholesterol *O*-acyltransferase (ACAT), lecithin: cholesterol *O*-acyltransferas (LCAT), or cholesterol esterase).[22] This is the first example elucidated of a lipophilic conjugate causing toxicity.[2]

METABOLISM IN HUMANS

In vitro comparative metabolism of ^{14}C-*trans*-permethrin labeled in the alcohol moiety and ^{14}C-*trans*-phenothrin labeled in the acid moiety were carried out in human and rat hepatic microsomes and revealed that both microsomes show similar HPLC radioautograms, indicating products derived from ester hydrolysis to be the major metabolites.[23] Others from oxidation reactions were relatively minor (Figure 6). Human and rat hepatic microsomes show comparable ability to hydrolyze ester linkages and oxidize various sites of pyrethroids, resulting in the formation of metabolites found in in vivo rat studies. Furthermore, we examined which CYP isoforms are responsible for the oxidation using 9 human and 14 rat CYP isoforms. It is shown that human CYP1A2, 2C19, 2C9, 2D6, 2E1, and 3A4 and rat CYP1A1, 2A1, 2C6, 2C11, 3A1, and 3A2 have relatively high activity for *trans*-permethrin and that human CYP2B6, 2C19, 2C9, and 2D6 and rat CYP1A1, 2C6, 2C11, and 3A2 are more active for *trans*-phenothrin.[23] These findings are generally similar to the results in other pyrethroids reported previously.[24,25] On the other hand, PBalc is more rapidly oxidized at the 4′-position of the ring by human CYP 2E1 than by human 2C19 and 2D6 and by rat CYP 2E1 than by rat CYP1A1, 2C6, and 2C11.[23] CYP2E1 is likely to play an important role in 4′-hydroxylation of the 3-phenoxybenzyl alcohol moiety.

SPECIES DIFFERENCES BETWEEN HUMANS AND LABORATORY ANIMALS

Metabolism studies of pyrethroids in humans have been comparatively limited, but the data obtained so far indicate that the

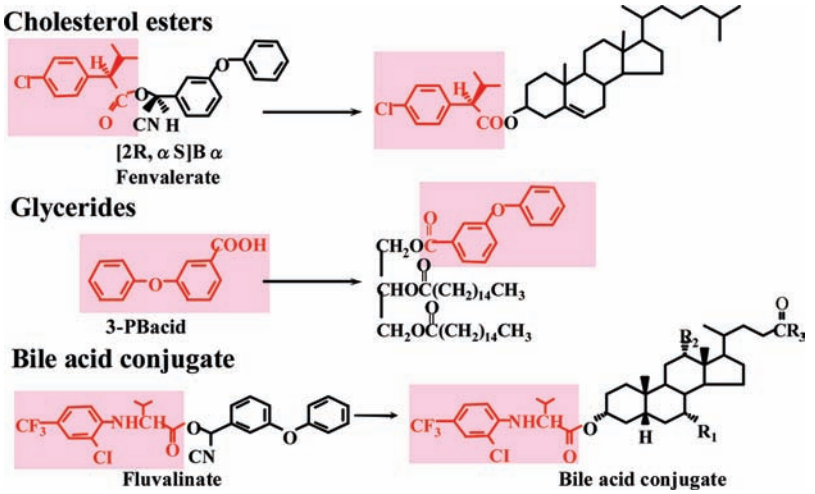

Figure 4. Metabolic reaction 4: lipophilic conjugates.

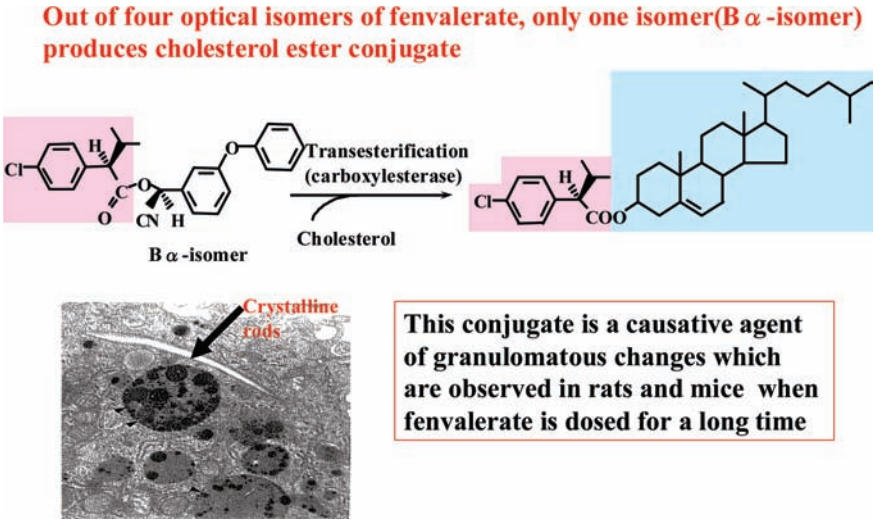

Figure 5. Metabolic differences among chiral isomers of fenvalerate-chiral specific formation of a cholesterol ester conjugate.

reactions of pyrethroids are similar to those in rodents.[26,27] That is, ester hydrolysis and oxidation are shared in common. However, it is reported that the absorption rate of pyrethroids through the skin is remarkably different between humans and rats and that humans show much less skin penetration with cypermethrin, indicating that rat models overestimate human skin penetration (i.e., penetration rate (%) of dose: human in vivo, 1.2%, versus rat in vivo, 12%, for 24 h).[28] This overestimation is similarly seen in dermal absorption studies of permethrin, bifenthrin, and deltamethrin.[29,30] With respect to the enzymes involved in the metabolism of pyrethroids, CYP2C9 and 3A4 in humans and 2C11 and 2C6 in rats seem to play predominant roles in oxidation reactions in terms of abundance and specific activity.[25] Major carboxylesterases for ester hydrolysis are hCE1 in humans and hydrolases A and B in rats, with a significant species difference found in serum esterase. Rat serum has high esterase activity for pyrethroids, whereas humans show substantially no activity.[24,31] What is important, however, is that it is likely that there are actually no poor metabolizers for pyrethroids in humans, because several CYP isoforms and carboxylesterase(s) are found to be involved.

TOXICITY

Pyrethroids show moderate acute oral toxicity, and typical toxicological signs are tremors for type I (generally pyrethroids without the CN group in the alcohol moiety) and choreoathetosis with salivation (CS symptoms) for type II form (generally pyrethroids with the CN group in the alcohol moiety). Some pyrethroids are reported to show mixed clinical signs.[2]

Teratogenicity and genotoxicity results have generally been negative;[32] however, carcinogenicity studies have shown some positive results. In some cases, the mode of action for carcinogenicity has been elucidated. As a typical example, metofluthrin can be illustrated.[33] This pyrethroid causes hepatocellular tumors in rats through nongenotoxic mechanisms involving CYP 2B induction, demonstrated to be mediated by nuclear hormone receptor CAR using RNAi technology. In addition, clustering analysis of transcriptomes shows similar results with both metofluthrin and phenobarbital. From the available results taken together, metofluthrin induction of rat hepatic tumors does not appear to be relevant to the human case, as also shown with phenobarbital.[33] Importantly, with regard to carcinogenicity, no

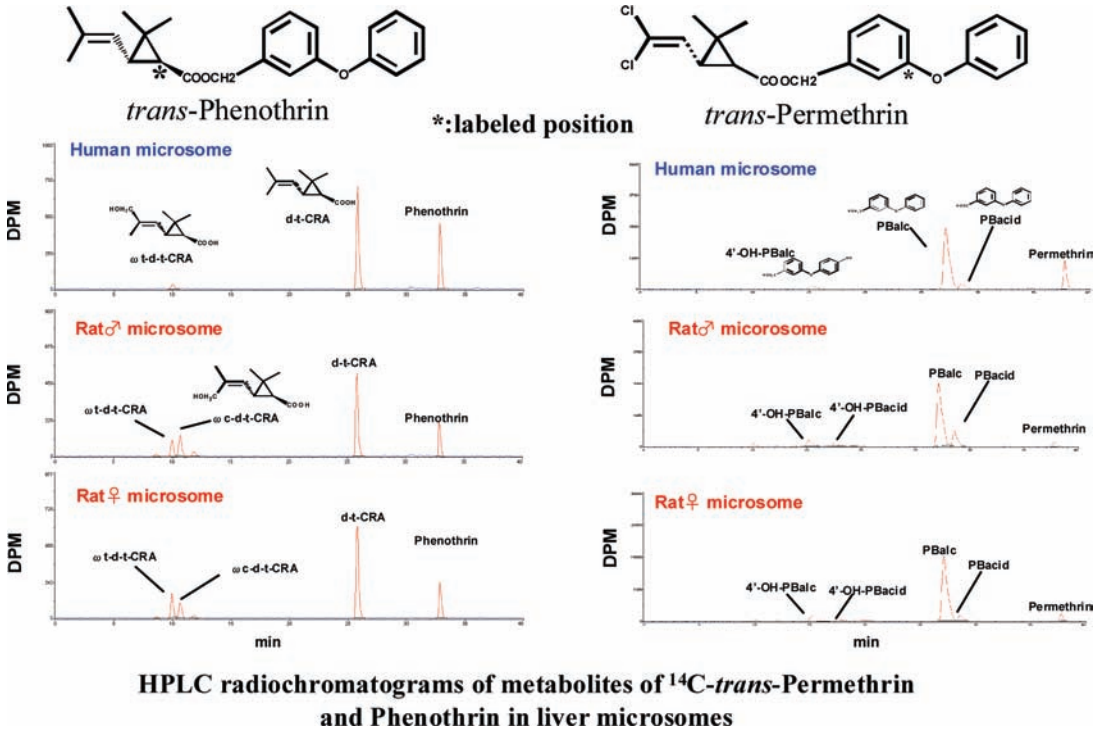

Figure 6. In vitro comparative metabolism in human and rat liver microsomes.

reports have appeared regarding cancer in humans following oral, dermal, or inhalation exposure to pyrethroids.[34] In addition, no reports were located regarding reproductive or developmental effects in humans following exposure to pyrethroids.[34]

CONCLUSIONS

Mammalian metabolism studies of many pyrethroid insecticides have been extensively carried out for more than 40 years; however, I think that there remain several important research fields such as in vitro or in vivo comparative studies of pharmacokinetics, age difference, metabolism in major tissues including liver, kidney, and intestine, and metabolic enzymes and transporters involved in pyrethroid metabolism between humans and rodents for better risk assessment and better extrapolation from animal data to human data. I believe that the above metabolism studies will lead to further confirmation that pyrethroids are one of the safest pesticide groups.

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