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In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and
cytochrome P450 isoforms

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Abstract

Species differences in the intrinsic clearance (CL_{int}) and the enzymes involved in the metabolism of pyrethroid pesticides were examined in rat and human hepatic microsomes. The pyrethroids bifenthrin, S-bioallethrin, bioresmethrin, β -cyfluthrin, cypermethrin, cis-permethrin and trans-permethrin were incubated in rat and human hepatic microsomes in the presence or absence of NADPH. Metabolism was measured using a parent depletion approach. The CL_{int} of the pyrethroids was 5- to 15-fold greater in rat relative to human microsomes except for trans-permethrin, which was about 45% greater in human microsomes. The metabolism of bifenthrin, S-bioallethrin and cis-permethrin in rat and human hepatic microsomes was due solely to oxidative processes. The metabolism of bioresmethrin and cypermethrin in human hepatic microsomes was due solely to hydrolytic processes. Bioresmethrin and cypermethrin in rat hepatic microsomes and β -cyfluthrin and trans-permethrin in microsomes from both species were metabolized by both oxidative and hydrolytic pathways. The metabolism of trans-permethrin was reduced when incubated with its diastereomer, cis-permethrin, in both rat and human hepatic microsomes. Rat P450 isoforms that showed activity towards several pyrethroids included CYP1A1, CYP1A2, CYP2C6, CYP2C11, CYP3A1 and CYP3A2. Human P450 isoforms that showed activity towards multiple pyrethroids were CYP2C8, CYP2C9, CYP2C19 and CYP3A4. Species specific differences in metabolism may result in variable detoxification of pyrethroids, which may in turn result in divergent neurotoxic outcomes. These species differences and isomer interactions in metabolism of pyrethroids should be considered when assessing the potential adverse health effects of pyrethroid pesticides.

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Introduction

Pyrethroids are synthetic insecticides structurally based on the pyrethrins, which are botanical insecticides (Soderlund et al., 2002). Pyrethrins are potent insecticides with relatively low mammalian toxicity, but are sensitive to air and light. Hence, the use of pyrethrins for crop protection and to control disease-carrying insects is limited. With an altered structure, the pyrethroids are more photostable, while retaining the insecticidal activity of the pyrethrins. Thus, pyrethroids are widely used today in agriculture and as medical and veterinary products.

The basic pyrethroid structure consists of an acid and alcohol moiety (Figure 1). Pyrethroids can possess 1-3 chiral centers resulting in 2-8 enantiomers. The centers of chirality are in the cyclopropane ring of the acid moiety (Figure 2) and the α -carbon of the alcohol moiety (Figure 1). Each enantiomer may differ in insecticidal potency, mammalian toxicity and metabolic pathway. These pathways include NADPH-dependent oxidation and non-NADPH-dependent hydrolysis catalyzed by cytochrome P450s (P450) and esterases, respectively (Anand et al., 2006a; Crow et al., 2007; Godin et al., 2006, 2007; Nishi et al., 2006; Ross et al., 2006).

Pyrethroids can be classified as Type I or Type II compounds (Soderlund et al., 2002). Type I pyrethroids are esters of primary or secondary alcohols. Type II pyrethroids are esters of secondary alcohols with a cyano group at the α -carbon of the alcohol moiety (Figure 1). Soderlund and Casida (1977), using mouse liver microsomes, examined how the structure of a pyrethroid influences its metabolic pathway. Of the Type I pyrethroids examined, the trans isomers were the most rapidly metabolized, primarily by hydrolysis. The cis isomers were metabolized at a relatively slower rate,

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primarily by oxidation. The Type II pyrethroids studied had the lowest overall metabolic rate, and the metabolism generally occurred by both pathways.

The signs of acute toxicity in rats differ between Type I and Type II pyrethroids (Ray and Forshaw, 2000; Soderlund et al., 2002). Type II pyrethroids are generally more potent than Type I pyrethroids. Symptoms in rats administered Type I pyrethroids include aggression and hypersensitivity, general and fine tremor, convulsive twitching, coma and death. Type II pyrethroids elicit pawing and burrowing, salivation, coarse tremor, increased extensor tone, writhing convulsions and death. The site of action of pyrethroids is the voltage-dependent sodium channel, but the chloride, calcium and other channels may also be targets (Ray and Forshaw, 2000; Soderlund et al., 2002; Shafer and Meyer, 2004; Ray and Fry, 2006).

The parent pyrethroid is generally believed to be the neurotoxic entity (Soderlund et al., 2002), as metabolism decreases its potency. The characteristic neurotoxic effects of the pyrethroid resmethrin observed in rats following oral administration were not observed after intravenous administration of its hydrolyzed products, *cis*- and *trans*-chrysanthemic acid or 5-benzyl-3-furylmethyl alcohol (White et al., 1976). Intracerebral administration of several pyrethroids in mice confirmed the neurotoxic findings following their intraperitoneal administration in mice and oral or intravenous administration in rats (Lawrence and Casida, 1982). Levels of deltamethrin, a Type II pyrethroid, in rat brain correlate with the onset of neurotoxicity in this species (Rickard and Brodie, 1985). More recently, Anand et al. (2006b) reported that the severity of neurotoxicity of deltamethrin was greater in 10- than in 40-day-old rats because of its limited metabolism

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in the younger animals. Pyrethroid metabolism is an important determinant of the toxicological outcome following exposure.

Pyrethroids are generally formulated as a mixture of the isomers. The possibility exists that enantiomers or diastereomers of a pyrethroid may interact metabolically or dynamically. This interaction could lead to several outcomes. Since the metabolism of pyrethroids is believed to be a detoxification process, the most adverse outcome of this isomer interaction would be competitive inhibition of the metabolism of the most neurotoxic isomer. This could lead to enhanced neurotoxicity. If competitive metabolic inhibition by the isomers occurs, it would be expected to be dose-dependent. This may be a high dose effect, which may occur occupationally but most likely not following environmentally low exposures to pyrethroids.

The objective of this study was to determine the intrinsic clearance, a measurement of metabolic rate, of several Type I and II pyrethroids in rat and human hepatic microsomes, to assess the relative role of oxidative and hydrolytic pathways of these pyrethroids, to investigate the potential interaction of diastereomers of permethrin at the metabolic level and to screen rat and human P450 isoforms for their ability to metabolize pyrethroids. A better understanding of the metabolic differences between rodents and humans may allow for a more accurate extrapolation of the rodent pyrethroid toxicity data to human exposures.

Methods

Chemicals. The pyrethroids bifenthrin ((2-methyl[1,1'-biphenyl]-3-yl)methyl (1*R*,3*R*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-

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dimethylcyclopropanecarboxylate) (100% cis), S-bioallethrin (2-methyl-4-oxo-3-(2-propenyl)-2-cyclopenten-1-yl (1*R*,3*R*)-2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate) (100% trans), bioresmethrin ([5-(phenylmethyl)-3-furanyl]methyl (1*R*,3*R*)-2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate) (96% trans: 2% cis), β -cyfluthrin (cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) (67% trans:33% cis), γ -cyhalothrin ((*R*)-cyano(3-phenoxyphenyl)methyl (1*S*,3*S*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate) (100% cis), cypermethrin (cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) (49% cis:51% trans), cis-permethrin ((3-phenoxybenzyl (1*RS*)-*cis*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) (100% cis), trans-permethrin ((3-phenoxybenzyl (1*RS*)-*trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) (96% trans:4% cis), and resmethrin ([5-(phenylmethyl)-3-furanyl]methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate) (30% cis:70% trans) were purchased from Chem Service Inc. (West Chester, PA). Structures of the pyrethroids used in this study are shown Figure 3. Purity exceeded 98% for all pyrethroids except trans-permethrin (94% trans and 4% cis) and bioresmethrin (96% trans and 2% cis). The commercial formulation of permethrin (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) (40:60, cis:trans) was a gift from FMC Corporation (Philadelphia, PA). Labeled cis- and trans-permethrin (phenoxy-¹³C₆) were purchased from Cambridge Isotope Laboratories (Andover, MA). These pyrethroids were chosen to study because they are registered for use on food crops. Solvents, including acetone,

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hexanes, pentane (Fisher Scientific, Pittsburgh, PA) and methanol (VWR, West Chester, PA) were pesticide grade. Sucrose, ethylenediaminetetraacetic acid (disodium salt dihydrate) (EDTA), KCl, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (sodium salt) (NaHepes), glycerol, dithiothreitol, Trizma-base and β -nicotinamide adenine dinucleotide phosphate reduced form (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol (pesticide grade) was purchased from Burdick and Jackson (Morristown, NJ). Ammonium acetate was from Fluka Chemicals (St. Louis, MO). Unless otherwise specified, chemicals used were of the highest grade commercially available.

Animals. Long Evans rats (male, approximately 70 days old, 275-299 g) were purchased from Charles River Laboratories (Raleigh, NC) and allowed to acclimate for a minimum of four days in an Association for the Assessment and Accreditation of Laboratory Animal Care approved facility. The Long Evans rat was used because of ongoing collaborations that are investigating the neurotoxicity of pyrethroids in this strain (Wolansky et al., 2006). The overall goal of our collaborative project is to develop biologically-based dose response models for the pyrethroids. Therefore, we found it necessary in our metabolism studies to use the same rat strain as that used in the neurotoxicity studies. All animal procedures were approved by the Institutional Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory. Rats were housed in pairs in polypropylene cages lined with heat-treated pine shavings. Temperature, humidity and photoperiod were maintained at 21 ± 2 °C, $50 \pm 10\%$ and 12 h light:12 h dark, respectively. Feed (Purina Rodent Chow 5001, Barnes Supply Co., Durham, NC) and tap water were provided ad libitum.

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Rat hepatic microsomal preparation. Rat hepatic microsomes were a pool of livers from six animals. Three pools of microsomes were prepared using a total of 18 animals. Pools of microsomes were prepared because of our interest in comparing metabolism data across a series of environmental chemicals, including pyrethroid pesticides and minimizing experimental variability. Our concern was not that of assessing inter-animal variability. Rats were anesthetized with CO₂ and sacrificed via cardiac puncture. Livers were removed, weighed and homogenized (1:4 w/v) in ice-cold buffer containing 250 mM sucrose, 0.5 mM EDTA, 0.25 mM KCl, 10 mM NaHepes, 1 mM dithiothreitol and 10% glycerol. Homogenates were centrifuged at 12,000 x g for 21.5 minutes in a Beckman Instruments (Palo Alto, CA) J2-21M Induction Drive Centrifuge using a JA-17 rotor. The supernatant was removed and centrifuged at 105,000 x g for 60 min in a Beckman Instruments XL-80 Ultracentrifuge using a 50.2 Ti rotor. The cytosolic supernatant was discarded. The pellet was washed by resuspending it in homogenization buffer and centrifuging for a second time at 105,000 x g for 60 min. After the wash, the pellet was resuspended in 25 ml of homogenization buffer. Microsomal protein was quantified using the Bio-Rad protein assay (Bio-Rad, Hercules, CA) standardized with bovine serum albumin. Microsomes were stored at -80 °C until use.

Human microsomes. Pooled human adult liver microsomes were purchased from CellzDirect (Phoenix, AZ; lot number HMMC-PL020, a mixed gender pool of 15), Cedra (Austin, TX, lot number 821-2, a mixed gender pool of 15) and Xenotech (Lenexa, KS, lot number 051022, a mixed gender pool of 50). Using human microsomes from three different sources limited the potential bias in preparation and processing the

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samples. Each pool of microsomes was used in a separate experiment. Microsomes were stored at -80 °C until use.

Rat and Human P450s. Supersomes™ (microsomes) containing specific rat (CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C6, CYP2C11, CYP2C12, CYP2C13, CYP2D1, CYP2D2, CYP3A1 and CYP3A2) or human (CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9*1, CYP2C9*2, CYP2C9*3, CYP2C19 and CYP3A4) P450 isoforms were purchased from BD Biosciences (Woburn, MA). The isoform is expressed from a rat or human P450 cDNA using a baculovirus-infected cell system. The microsomes prepared from this system also contain P450 reductase. The P450 content ranged from 1000 to 2000 pmol/ml.

Determination of K_m and V_{max} in rat hepatic microsomes. Estimates of K_m and V_{max} were determined in rat hepatic microsomes for bifenthrin, S-bioallethrin, bioresmethrin, cis-permethrin, trans-permethrin and a 40:60 cis:trans permethrin mixture. This determination was done to ensure pyrethroid concentrations in the parent depletion assays described below were $\ll K_m$. The assays were conducted in duplicate for each of the three pools of microsomes (N=3) by measuring the disappearance of parent compounds over 10 min. Each assay tube consisted of 3.5 ml of 0.1 M Trizma buffer (pH 7.4), 1 mg/ml microsomal protein and 1 mg/ml NADPH. This mixture was preincubated at 37 °C for 10 min. Assays were initiated by the addition of pyrethroid in 25 μ l methanol at time 0 to attain final pyrethroid concentrations of 0.1, 1, 5, 10, 20 or 50 μ M. At time 0, 2, 6, 8 and 10 min, 250 μ l aliquots were removed from the mixture and dispensed into silanized 16 x 100 mm culture tubes containing 2 ml ice-cold 5% acetone:pentane. To assess analyte recovery, 50 ng of trans-permethrin ($^{13}\text{C}_6$ -phenoxy)

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in methanol were added to the solvent/sample mixtures as a surrogate. Parent pyrethroids were extracted from the buffer by vortexing for 5 min followed by centrifuging at 1300 x g for 5 min. The tubes were placed into a methanol/dry ice bath to freeze the aqueous layer. The unfrozen organic solvent was poured into 13 x 100 mm culture tubes. The extraction was repeated twice more with 2 ml of 5% acetone:pentane each time. Extracts were combined and dried under a stream of nitrogen. Samples were reconstituted in 1 ml of methanol. As an internal standard, 50 ng of cis-permethrin ($^{13}\text{C}_6$ -phenoxy) in methanol were added to each sample. Control incubations were performed in Trizma buffer with NADPH in the absence of microsomal protein to ensure that metabolism of the pyrethroids was enzymatic. There was no significant loss of pyrethroid in the control incubations. Concentration of substrate was determined by HPLC-MS/MS analysis (see below for details) over the time course and converted to moles of substrate remaining. Substrate remaining was converted to product formed and plotted versus time to produce a reaction velocity. Plotting reaction velocities against initial concentrations yielded K_m and V_{max} estimates (JMP ver. 6, SAS Institute, Cary, NC) for each pyrethroid.

Determination of metabolic rate constants in rat and human microsomes. In vitro metabolic rate constants were determined for bifenthrin, S-bioallethrin, bioresmethrin, β -cyfluthrin, cypermethrin, cis-permethrin and trans-permethrin in rat and human hepatic microsomes. A 0.5 μM concentration for the pyrethroids was used except for S-bioallethrin (1 μM). The K_m s for the pyrethroids were determined as described above with the rat hepatic microsomes, except for β -cyfluthrin and cypermethrin. The K_m values ranged from 3.7 – 31.1 μM . Based on these results, we assumed that a concentration of 0.5 μM for β -cyfluthrin and cypermethrin was below the rat hepatic

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microsomal K_m s for these two compounds. A similar assumption was made for the pyrethroid concentrations used in the human hepatic microsomal incubations.

Microsomal metabolism assays with the pyrethroids were conducted using the parent compound depletion approach as described above. Using this approach, only the metabolic loss of parent chemical is quantitated. This approach was taken in part because it is generally believed the parent pyrethroid is the neurotoxic entity, whereas its metabolism results in detoxification of the compound. Briefly, pyrethroid in 25 μ l methanol was added to a 1 ml incubation mixture (37 °C) of 0.1 M Trizma buffer (pH 7.4), 1 mg/ml microsomal protein and 1 mg/ml NADPH. At 0, 4 and 8 min, 250 μ l aliquots of incubate were removed and handled as described above. Assays to assess the microsomal hydrolysis of the pyrethroids were conducted as described above but in the absence of NADPH. Each incubation, with or without NADPH, was conducted in duplicate using the three different pools of microsomes (N=3).

Calculation of metabolic rate constants and intrinsic hepatic clearance rates.

First-order metabolic rate constants (k) were determined from the linear regression of the natural log of the percentage pyrethroid remaining versus time. The slope of the regression line equals k (min^{-1}). At substrate concentrations significantly below K_m , the clearance of a chemical is constant (Iwatsubo et al., 1997) and can be calculated using k (Obach, 1999). Intrinsic clearance (CL_{int}) was estimated using the following equation:
 $CL_{int} = k$ (min^{-1}) \cdot ml incubation/mg microsomes \cdot mg microsomes/g liver \cdot g liver/kg body weight (Obach et al., 1997; Obach, 1999).

Metabolism of pyrethroids by rat and human P450 isoforms. The capacity of selected rat and human P450 isoforms to metabolize bifenthrin, S-bioallethrin,

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bioresmethrin, β -cyfluthrin, λ -cyhalothrin, cypermethrin, cis-permethrin, trans-permethrin and resmethrin was examined. Briefly, 10 μ l of a 50 μ M pyrethroid stock solution (final concentration, 0.5 μ M) was added to a 1 ml incubation (37 °C) mixture containing 0.1 M Trizma buffer (pH 7.4), supersomes containing 10 pmol P450 and 1 mg/ml NADPH. Each incubation was conducted in triplicate (N=3). At 0 and 10 min, 250 μ l aliquots were removed from the mixture and handled as described above and analyzed for parent compound.

Analysis and Quantification of Pyrethroids. An Applied Biosystems (Foster City, CA) API 4000 LC/MS/MS fronted with an Agilent (Santa Clara, CA) 1100 Series liquid chromatography system was used to analyze the pyrethroids. Isocratic elution of the pyrethroids off an Agilent Zorbax Eclipse XDB-C18 column (3 x 150 mm, 3.5 μ m) was accomplished with a 98%:2% methanol:5 mM ammonium acetate solution flowing at 400 μ l/min. The mass spectrometer was operated with the turbo ion spray/atmospheric pressure ionization source. Positive ionization was used for all compounds and the analytes were identified using transition ion pairs and retention times.

Pyrethroid concentration was calculated using a five-point calibration curve, prepared in sample matrix containing cis-permethrin ($^{13}\text{C}_6$ -phenoxy) as an internal standard. The concentrations of the extracted pyrethroids were determined by the ratio of internal standard response to analyte response. Calibration standards ranged from 0.01-0.6 μ M. However, curves were generally limited to two orders of magnitude. Samples with chemical concentrations outside the calibration curve were reanalyzed with an appropriate curve. In addition, samples with surrogate concentrations of trans-permethrin ($^{13}\text{C}_6$ -phenoxy) that varied greater than 20% of expected concentrations were reanalyzed.

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Method recoveries were determined by spiking preincubated (37 °C) microsomal preparations, excluding NADPH, with individual pyrethroids at a final concentration of 0.5 µM. The incubations were vortexed for 5 seconds and immediately extracted as described previously. Percent recoveries and coefficients of variations were 84-108% and 5-10%, respectively (Table 1).

Statistical analysis. A one sample t-test was used to assess whether pyrethroid clearance in the absence of NADPH was significantly different from zero ($p < 0.05$) (GraphPad InStat, version 3.05, GraphPad Software, San Diego, CA). An unpaired t-test was used to assess whether the pyrethroid clearance values in the presence or absence of NADPH were significantly different ($p < 0.05$). The percent metabolism of pyrethroids by rat and human P450s was assessed by a one sample t-test to assess if the value was significantly different from zero ($p < 0.05$).

Results

Estimates of K_m and V_{max} for Type I pyrethroids in rat hepatic microsomes. The K_m s ranged from 3.7 (cis-permethrin) to 31.1 µM (S-bioallethrin) with respective 95% confidence intervals of 1.6-9.2 and 8.38-233.2 µM (Table 2). A similar trend was observed for V_{max} with cis-permethrin having the lowest (0.14 nmol/min/mg microsomal protein) and S-bioallethrin the highest (4.4 nmole/min/mg microsomal protein) value. The relative ranking from lowest to highest K_m and V_{max} was cis-permethrin < bioresmethrin < bifenthrin < trans-permethrin < S-bioallethrin. Hepatic clearance values, estimated by V_{max}/K_m , were ranked as: cis-permethrin < bioresmethrin < trans-permethrin \approx bifenthrin < S-bioallethrin.

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The K_m and V_{max} of the permethrin isomers (40:60, cis:trans) in the mixture (Table 3) were similar to the values when cis- or trans-permethrin were incubated alone (Table 2). The hepatic clearance (V_{max}/K_m) estimates for cis- and trans-permethrin in the mixture (Table 3) were greater than the estimates in the single isomer experiments (Table 2). The differences are largely accounted for by the greater V_{max} for cis-permethrin and lower K_m for trans-permethrin in the mixture. The sum of cis- and trans-permethrin concentrations was used to calculate the total permethrin metabolic parameters. The total permethrin K_m was lower than the values for the cis and trans isomers. The total permethrin V_{max} was two times greater than the cis value.

Intrinsic clearance of pyrethroids from rat and human hepatic microsomes. The intrinsic clearance (CL_{int}) values of the Type I pyrethroids examined in rat hepatic microsomes, with one exception, exceeded those in human microsomes (Table 4). Except for trans-permethrin, the CL_{int} of these pyrethroids was 5- to 15-fold greater in rat relative to human hepatic microsomes. The CL_{int} of trans-permethrin was 45% greater in human than in rat microsomes. The pyrethroids with the highest CL_{int} in rat and human microsomes were of the trans configuration. These pyrethroids are bioresmethrin and trans-permethrin, respectively. In rat microsomes, the rank order from highest to lowest CL_{int} was bioresmethrin, S-bioallethrin, cis-permethrin, trans-permethrin and bifenthrin. In human microsomes, the rank order was trans-permethrin, bioresmethrin, S-bioallethrin, cis-permethrin and bifenthrin.

The contributions of oxidative and hydrolytic pathways were determined by comparing the metabolism of the pyrethroids in the presence or absence of NADPH. The contributions of the oxidative mechanisms were calculated by subtracting the non-

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NADPH (or hydrolytic) contribution from the overall metabolic rate. The metabolism of bifenthrin, S-bioallethrin and cis-permethrin was attributed to the oxidative pathway because they lacked significant metabolism in the absence of NADPH (Table 4). Trans-permethrin was metabolized by both oxidative and hydrolytic processes in rat and human hepatic microsomes. However, hydrolysis of trans-permethrin was greater in rat microsomes while oxidative metabolism predominated in human microsomes. The metabolism of bioresmethrin by rat hepatic microsomes was a combination of oxidative and hydrolytic mechanisms, similar to trans-permethrin. However, in human microsomes, NADPH-dependent metabolism of bioresmethrin was limited.

The CL_{int} of β -cyfluthrin and cypermethrin, determined by summation of all co-occurring isomers, was greater in the rat relative to human hepatic microsomes (Table 5). However, the differences in CL_{in} between rat and human microsomes were much smaller than those of the Type I pyrethroids (Table 5), varying by a factor of three. Cypermethrin and β -cyfluthrin are primarily metabolized through oxidative mechanisms in rat and hydrolytic mechanisms in human hepatic microsomes.

Intrinsic clearance of a permethrin mixture. The impact of incubating a mixture of pyrethroid isomers on the CL_{int} of the individual isomers was examined using permethrin (40:60, cis:trans). The CL_{int} of cis-permethrin incubated as either an individual isomer or in the mixture, was 10-fold greater or more in rat than in human hepatic microsomes (Table 6). Trans-permethrin was cleared more quickly by human than rat hepatic microsomes when incubated as an individual isomer. The CL_{int} of trans-permethrin was similar between microsomes from both species when incubated in the mixture. However, the CL_{int} of trans-permethrin in the mixture decreased by 40% in rat

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and 64% in human hepatic microsomes. The CL_{int} of total permethrin was approximately 3-fold greater in rat than in human hepatic microsomes.

Metabolism of pyrethroids by rat and human P450s. Nine pyrethroids (6 Type I, 3 Type II) were screened for metabolism in the presence of NADPH by twelve rat and nine human P450s (Tables 7 and 8). Overall, more rat P450 isoforms had the ability to metabolize these pyrethroids to a significant extent than the human P450 isoforms. With the rat P450s, setting a level of 25% or more metabolism of parent pyrethroid in 10 min, the isoforms that showed activity were CYP1A1 (9/9 pyrethroids), CYP1A2 (3/9), CYP2C6 (9/9), CYP2C11 (8/9), CYP2D1 (1/9), CYP3A1 (8/9) and CYP3A2 (6/9). With the human P450s, the isoforms that showed activity were CYP1A1 (1/9 pyrethroids), CYP1A2 (1/9), CYP2B6 (1/9), CYP2C8 (3/9), CYP2C9*1 (4/9), CYP2C9*2 (2/9), CYP2C19 (9/9) and CYP3A4 (2/9).

Discussion

Rodents have in general a higher xenobiotic metabolic rate than humans. This is evident in the present study as the CL_{int} of six of the seven pyrethroids examined was greater in rat than in human hepatic microsomes. The greater CL_{int} in rat hepatic microsomes may be explained by greater abundance, specificity or catalytic activity of the pyrethroid metabolizing enzymes than in human hepatic microsomes. Hepatic P450s have a major role in the metabolism of pyrethroids. The hepatic P450 content is greater in rats than humans when expressed either in terms of g of liver (2.5- to 3-fold greater) or mg of microsomal protein (1.5-fold greater) (Kremers et al., 1981). Hepatic and extra-hepatic carboxylesterases metabolize pyrethroids (Anand et al., 2000a; Crow et al., 2007;

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Godin et al., 2006; Nishi et al., 2006; Ross et al., 2006). The in vitro hydrolysis of esfenvalerate is approximately 2-fold greater for rat hydrolase A than human carboxylesterase-1, two abundant hepatic carboxylesterases (Godin et al., 2006). However, there are exceptions, in that the CL_{int} of trans-permethrin (present study) and deltamethrin (Godin et al., 2006), was greater in human than rat hepatic microsomes. While the metabolic rate of xenobiotics is typically greater for rodents, at a minimum, in vitro experiments should be conducted to assure that this hypothesis is correct. If assumed, misleading extrapolations from rodent to humans may occur in risk assessments.

A species difference in the two major enzymatic pathways of in vitro pyrethroid metabolism was detected. Metabolism of bioresmethrin and cypermethrin in human hepatic microsomes did not require NADPH, suggesting that it was solely by hydrolysis. In rat hepatic microsomes, metabolism of these two pyrethroids was a combination of oxidation and hydrolysis, with the former pathway being predominant. Similar results have been reported with deltamethrin in human and rat microsomes (Anand et al., 2006a; Godin et al., 2006). The qualitative and quantitative differences between rat and human hepatic microsomes in pyrethroid metabolism suggests that the sole use of rat metabolism data in the development of pharmacokinetic models of this pesticide for human extrapolation should be approached guardedly.

There is a general but not exact relationship between pyrethroid structure and its pathway and rate of metabolism. In mouse liver microsomes, primary alcohol esters of trans-substituted cyclopropanecarboxylic acids are the most rapidly metabolized pyrethroids, primarily by hydrolysis (Soderlund and Casida, 1977). Bioresmethrin and

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trans-permethrin have this structure, but do not follow the same metabolic pathway in microsomes. Bioresmethrin was only metabolized by hydrolysis in human hepatic microsomes. However, in rat hepatic microsomes, oxidation of bioresmethrin was slightly greater than hydrolysis. With trans-permethrin, hydrolysis was slightly greater and lower than oxidation in rat and human hepatic microsomes, respectively. Soderlund and Casida (1977) reported that pyrethroids with primary alcohol esters of cis-substituted cyclopropanecarboxylic acids are metabolized slower than their trans-substituted isomers. This was evident with cis- and trans-permethrin in rat and human microsomes. Pyrethroids with a secondary alcohol ester tended to be metabolized by oxidation in mouse liver microsomes (Soderlund and Casida, 1977). S-bioallethrin has this structure and likewise, in rat and human hepatic microsomes, was only metabolized by oxidation. Pyrethroids with a cyano group on the α -carbon of the alcohol moiety (Type II compound) were metabolized the slowest in mouse hepatic microsomes and with a few exceptions, there was no clear preference in their metabolic pathway (Soderlund and Casida, 1977). In the present study, the metabolic rate of the Type II pyrethroids examined, β -cyfluthrin and cypermethrin, was on the lower end of the spectrum. There were clear species differences in their microsomal metabolic pathways. Oxidation was predominant in rat hepatic microsomes and hydrolysis was predominant in human hepatic microsomes for both pyrethroids. Similar results were reported with the Type II pyrethroid deltamethrin (Godin et al., 2006). However, the Type II pyrethroid esfenvalerate, which lacks a cyclopropane ring, was metabolized primarily by oxidation in both rat and human microsomes (Godin et al., 2006). This suggests that Type II

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pyrethroids with a cyclopropane ring are more susceptible to hydrolysis than oxidation in human hepatic microsomes.

Several rat and human P450 isoforms consistently metabolized the pyrethroids screened in the present study. Rat CYP 1A1 was very active towards the pyrethroids. However, 1A1 is expressed at low levels in rat liver (Martignoni et al., 2006) and is unlikely to have a major role in metabolizing pyrethroids. In rat small intestine, CYP1A1 is expressed more than in the liver (Zhang et al., 1996). Thus in rat small intestine, 1A1 may metabolize pyrethroids and limit their absorption. The most abundant P450 family in rat liver is 2C, with 2C11 being the predominant isoform in male rat (Martignoni et al. 2006). Of the rat isoforms, 2C11 and 2C6 had the most activity towards the pyrethroids screened. CYP2C11 is most likely the predominant isoform that metabolizes pyrethroids in male rat liver. In human liver, about 20 and 30% of total P450 is comprised of the CYP 2C and 3A families, respectively (Shimada et al., 1994). Isoforms 2C8 and 2C9 are the major forms of the 2C family in human liver, whereas 2C19, which has the greatest activity towards the pyrethroids, is expressed to a minor extent (Martignoni et al., 2006). In human liver, 3A4 and 2C9 are most likely the isoforms which metabolize the pyrethroids. A potential explanation for the relatively low mammalian pyrethroid toxicity is that there are several P450 isoforms in the liver and other tissues that metabolize this class of pesticides to less toxic forms. This is in addition to the activity of carboxylesterases towards the pyrethroids.

Pyrethroids are metabolized not only in liver microsomes, but also in hepatic cytosol, serum (or plasma) and small intestinal microsomes (Anand et al., 2006a; Crow et al., 2007; Godin et al., 2007). Rat and human hepatic cytosol are approximately 2-fold

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less hydrolytically active towards trans-permethrin than the corresponding microsomes (Crow et al., 2007). Human small intestinal microsomes hydrolyzed trans-permethrin, but not deltamethrin or bioresmethrin (Crow et al., 2007). Hydrolysis of trans-permethrin is slower in rat than in human small intestinal microsomes (Crow et al., 2007).

Deltamethrin is hydrolyzed in rat serum and plasma (Anand et al., 2006a; Godin et al., 2007). However, deltamethrin is not metabolized in human serum (Godin et al, 2007), because it lacks carboxylesterase activity (Li et al., 2005). Because hepatic cytosol has pyrethroid hydrolytic activity, it may be necessary to take this into account along with hepatic microsomal activity for a better estimate of CL_{int} . Finally, the observation that rat serum (plasma) is hydrolytically active towards the pyrethroids, whereas human serum is not, needs to be considered when extrapolating metabolic results from rat to human.

Many pyrethroids are formulated as a mixture of isomers. Permethrin consists of two diastereomers (cis and trans) and 4 enantiomers. Pyrethroid isomers display different toxic potencies which may be due to isomer-specific pharmacodynamic and pharmacokinetic processes. For example, in male mice the acute oral LD_{50} of (+)-cis-permethrin is 107 mg/kg, but is >5000 mg/kg for (-)-cis-permethrin (Miyamoto, 1976). Pyrethroid isomers may interact at their site of metabolism and toxicity. This interaction could alter the toxicity of a mixture of isomers as compared to a single isomer formulation. Results from this study suggest that cis-permethrin interacts with metabolism of trans-permethrin. The CL_{int} of trans-permethrin in the presence of cis-permethrin was decreased 40-64% relative to its incubation as a single isomer in rat and human hepatic microsomes. The CL_{int} of cis-permethrin was not similarly affected by trans-permethrin in either rat or human hepatic microsomes. Because trans-permethrin is

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hydrolyzed more readily in rat hepatic microsomes, this effect is not as great as in human hepatic microsomes. In human hepatic microsomes, the metabolism of trans-permethrin is more oxidative than hydrolytic. As the metabolism of cis-permethrin is predominantly oxidative in this fraction, and thus slower, it may interact with trans-permethrin and decrease its metabolism, perhaps by competitive inhibition. Other than human P450 isoforms 2C9*1 and 3A4 and rat 3A1 for cis-permethrin and human 2C9*2 for trans-permethrin, the P450 isoforms screened were fairly consistent in metabolizing these two diastereomers. This in vitro effect on the CL_{int} of pyrethroid isomers may potentially impact exposure-dose-response relationships in the risk assessment of commercial mixtures of pyrethroids.

In summary several pyrethroids were metabolized at rates greater in rat than in human hepatic microsomes. Overall, the pyrethroids examined were metabolized by oxidation and hydrolysis. However, bifenthrin, S-bioallethrin and cis-permethrin were metabolized predominantly by oxidation in both rat and human hepatic microsomes. In contrast, bioresmethrin and cypermethrin were metabolized primarily by hydrolysis in human hepatic microsomes. A metabolic interaction of cis- and trans-permethrin appears to have decreased the CL_{int} of trans-permethrin. Of the P450 isoforms examined, rat CYP1A1, CYP2C6, CYP2C11, CYP3A1 and CYP3A2 and human CYP2C19 have the most pyrethroid metabolizing activity. The potential for species differences and isomer interaction in the metabolism of pyrethroids should be considered in the toxicity assessment and development of pharmacokinetic and pharmacodynamic models for this class of pesticides.

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References

- Anand SS, Bruckner JV, Haines WT, Muralidhara S, Fisher JW, and Padilla S (2006a)
Characterization of deltamethrin metabolism by rat plasma and liver microsomes.
Toxicol Appl Pharmacol **212**:156-166.
- Anand SS, Kim K-B, Padilla S, Muralidhara S, Kim HJ, Fisher JW, and Bruckner JV
(2006b) Otogeny of hepatic and plasma metabolism of deltamethrin in vitro: role
in age-dependent acute neurotoxicity. *Drug Metab Dispos* **34**:389-397.
- Crow JA, Borazjani A, Potter PM, and Ross MK (2007) Hydrolysis of pyrethroids by
human and rat tissues: examination of intestinal, liver and serum
carboxylesterases. *Toxicol Appl Pharmacol* **221**:1-12.
- Godin SJ, Scollon EJ, Hughes MF, Potter PM, DeVito MJ, and Ross MK (2006)
Species differences in the in vitro metabolism of deltamethrin and esfenvalerate:
differential oxidative and hydrolytic metabolism by humans and rats. *Drug
Metab Dispos* **34**:1764-1771.
- Godin SJ, Crow JA, Scollon EJ, Hughes MF, DeVito MJ, and Ross MK (2007)
Identification of rat and human cytochrome P450 isoforms and a rat serum
esterase that metabolize the pyrethroid insecticides deltamethrin and
esfenvalerate. *Drug Metab Dispos* **35**:1664-1671.
- Iwatsubo T, Hirota N, Ooie T, Suzuzki H, Shimada N, Chiba K, Ishizuki T,
Green CE, Tyson CA, and Sugiyama Y (1997) Prediction of in vivo drug
metabolism in the human liver from in vitro metabolism data. *Pharmacol Ther*
73:147-171.

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Kremers P, Beaune P, Cresteil T, De Graeve J, Columelli S, Leroux J-P, and Gielen

JE (1981) Cytochrome P-450 monooxygenase activities in human and rat liver microsomes. *Eur J Biochem* **118**:599-606.

Lawrence LJ and Casida JE (1982) Pyrethroid toxicology: mouse intracerebral structure-toxicity relationships. *Pestic Biochem Physiol* **18**:9-14.

Li B, Sedelacek M, Manoharan I, Boopathy R, Duysen EG, Masson P, Lockridge O

(2005) Butyrylcholinesterase, paroxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. *Biochem Pharmacol* **70**:1673-1684.

Martignoni M, Groothuis GMM, and de Kanter R (2006) Species differences between

mouse, rat, dog, monkey and human cytochrome P450-mediated drug metabolism. *Expert Opin Drug Metab Toxicol* **2**:875-894.

Miyamoto J (1976) Degradation, metabolism and toxicity of synthetic pyrethroids.

Environ Health Perspect **14**:15-28.

Nishi K, Huang H, Kamita SG, Kim IH, Morisseau C, and Hammock BD (2006)

Characterization of pyrethroid hydrolysis by the human liver carboxylesterases hCE-1 and hCE-2. *Arch Biochem Biophys* **445**:115-123.

Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic

microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos* **27**:1350-1359.

DMD #22343

Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance

DJ, and Wastall P (1997) The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J Pharmacol Exp Ther* **283**:46-58.

Ray DE and Forshaw PJ (2000) Pyrethroid insecticides: poisoning syndromes, synergies, and therapy. *J Toxicol Clin Toxicol* **38**:95-101.

Ray DE and Fry JR (2006) A reassessment of the neurotoxicity of pyrethroid insecticides. *Pharmacol Ther* **111**:174-193.

Rickard J and Brodie ME (1985) Correlation of blood and brain levels of the neurotoxic pyrethroid deltamethrin with the onset of symptom in rats. *Pestic Biochem Physiol* **23**:143-156.

Ross MK, Borazjani A, Edwards CC, and Potter PM (2006) Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochem Pharmacol* **71**:657-669.

Shafer TJ and Meyer DA (2004) Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs and relationship to assessment of cumulative neurotoxicity. *Toxicol Appl Pharmacol* **196**:303-318.

Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270**:414-423.

DMD #22343

Soderlund DM and Casida JE (1977) Effects of pyrethroid structure on rates of hydrolysis and oxidation by mouse liver microsomal enzymes. *Pestic Biochem Physiol* **7**:391-401.

Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, and Weiner ML (2002) Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* **171**:3-59.

White INH, Verschoyle RD, Moradian MH, and Barnes JM (1976) The relationship between brain levels of cismethrin and bioresmethrin in female rats and neurotoxic effects. *Pestic Biochem Physiol* **6**:491-500.

Wolansky MJ, Gennings C, and Crofton KM (2006) Relative potencies for acute effects of pyrethroids on motor function in rats. *Toxicol Sci* **89**:271–277.

Zhang G-Y, Wikoff J, Dunbar D, and Kaminsky L (1996) Characterization of rat small intestinal cytochrome P450 composition and inducibility. *Drug Metab Dispos* **24**:322-328.

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Footnote

This article has been reviewed in accordance with the policy of the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Legends for Figures

Figure 1 Structure of the Type II pyrethroid cypermethrin. The basic pyrethroid structure consists of an acid and alcohol moiety. Type II pyrethroids also have a cyano group on the alpha-carbon of the alcohol moiety. For Type II pyrethroids, the alpha-carbon is a center of chirality.

Figure 2 Isomerism about carbon 1 and carbon 3 of the pyrethroid cyclopropane ring. The ring is drawn in the plane of the page. The substituent R_2 represents the alcohol moiety.

Figure 3 Structures of the pyrethroids used in this study. The structure of cypermethrin is shown in Figure 1. Bioresmethrin is the trans isomer of resmethrin (which is not shown).

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Table 1 % Recovery and coefficient of variation of pyrethroids extracted from rat hepatic microsomes

Pyrethroid	% Recovery	Coefficient of Variation
Bifenthrin	104	8
S-Bioallethrin	108	8
Bioresmethrin	100	9
β -Cyfluthrin	84	5
Cypermethrin	86	6
cis-Permethrin	98	10
trans-Permethrin	95	8
C ₁₃ -trans-Permethrin (surrogate standard)	108	5

Recoveries were determined by spiking preincubated (37 °C) microsomal preparations (1 mg/ml), excluding NADPH, with individual pyrethroids at a final concentration of 0.5 μ M. The incubations were vortexed for 5 sec and immediately extracted and analyzed by HPLC-MS/MS as described in the Methods. Five replicates were analyzed for each pyrethroid.

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Table 2 K_m , V_{max} and hepatic clearance (V_{max}/K_m) estimates for Type I
pyrethroids incubated in rat hepatic microsomes

Pyrethroid	Isomer	K_m^a	V_{max}^b	V_{max}/K_m^c
Bifenthrin	100% cis	5.42 (3.25 – 8.52) ^d	0.64 (0.56 – 0.74)	0.12
S-bioallethrin	100% trans	31.11 (8.38 – 233.16)	4.40 (2.81 – 12.59)	0.14
Bioresmethrin	96% trans 2% trans	3.87 (2.33 – 7.07)	0.32 (0.25 – 0.44)	0.08
cis-Permethrin	100% cis	3.70 (1.60 – 9.20)	0.14 (0.11 – 0.20)	0.04
trans-Permethrin	96% trans 4% cis	9.03 (5.66 – 15.42)	0.97 (0.82 – 1.23)	0.11

Values were obtained using three different pooled microsomal samples

^a μM

^b nmole/min/mg microsomal protein

^c ml/min/mg microsomal protein

^d 95% confidence interval

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Table 3 K_m , V_{max} and hepatic clearance (V_{max}/K_m) estimates for cis-permethrin, trans-permethrin and total permethrin incubated in rat hepatic microsomes as a 40:60 cis:trans permethrin mixture

Pyrethroid	K_m^a	V_{max}^b	V_{max}/K_m
cis-Permethrin	5.39 (3.11 – 10.39) ^c	0.41 (0.35 – 0.52)	0.08
trans-Permethrin	4.48 (1.80 – 12.86)	0.82 (0.61 – 1.25)	0.18
Permethrin – total	4.15 (1.87 – 10.11)	0.98 (0.76 – 1.37)	0.24

Values were obtained using three different pooled microsomal samples

^aμM

^bnmole/min/mg microsomal protein

^cml/min/mg microsomal protein

^c95% confidence interval

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Table 4 Intrinsic clearance (CL_{int}) and % oxidative and hydrolytic metabolism of Type I pyrethroids from rat and human hepatic microsomes

Pyrethroid	Isomer	Species	CL_{int}	% Oxidative	% Hydrolytic
Bifenthrin	100% cis	Rat	224 ± 20 ^a	100	NS ^b
		Human	20 ± 6	100	NS ^b
S-bioallethrin	100% trans	Rat	1218 ± 106	100	ND ^c
		Human	74 ± 5	100	ND ^c
Bioresmethrin	96% trans 2% cis	Rat	1515 ± 367	57	43
		Human	334 ± 102	NS ^d	100
cis-Permethrin	100% cis	Rat	529 ± 19	100	NS ^b
		Human	52 ± 12	100	NS ^b
trans-Permethrin	96% trans 4% cis	Rat	438 ± 46	35	65
		Human	636 ± 53	59	41

^avalues are ml/min/kg body weight ± SD, for three pooled microsomal samples
 microsomes

^bthe clearance value for hydrolysis (in the absence of NADPH) was not significantly different from zero as assessed by a one sample t-test, p<0.05

^cno metabolism was detected in the absence of NADPH

^dthe clearance values in the presence or absence of NADPH were not significantly different as assessed by an unpaired t-test, p<0.05

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Table 5 Intrinsic clearance (CL_{int}) and % oxidative and hydrolytic
metabolism of Type II pyrethroids from rat and human hepatic
microsomes

Pyrethroid	Isomer	Species	CL_{int}	% Oxidative	% Hydrolytic
β -Cyfluthrin	33% cis 67% trans	Rat	260 ± 27^a	81	19
		Human	89 ± 23	18	82
Cypermethrin	49% cis 51% trans	Rat	284 ± 17	85	15
		Human	104 ± 63	NS ^b	100

^avalues are ml/min/kg body weight, mean \pm SD, for three different pooled microsomal samples

^bthe clearance values in the presence or absence of NADPH were not significantly different as assessed by an unpaired t-test, $p < 0.05$

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Table 6 Intrinsic clearance of permethrin isomers incubated individually or as a mixture in rat and human hepatic microsomes

Species	Incubation condition	cis-Permethrin	trans-Permethrin	Total Permethrin
Rat	Individual Isomer	529 ± 19 ^a	438 ± 46	-
	Mixture 40:60 cis:trans	591 ± 32	267 ± 25	352 ± 20
Human	Individual Isomer	52 ± 12	636 ± 53	-
	Mixture 40:60 cis:trans	31 ± 13	226 ± 68	122 ± 25

^avalues are ml/min/kg body weight, mean ± SD, for three different pooled microsomal samples

Table 7 % Metabolism of Type I and Type II pyrethroids by rat P450 isoforms

Isoform	Bifenthrin	S-bioallethrin	Bioresmethrin	β -Cyfluthrin	λ -Cyhalothrin	Cypermethrin	cis-Permethrin	trans-Permethrin	Resmethrin
1A1	38.7 \pm 3.1 ^a	25.7 \pm 1.5	66.3 \pm 3.1	61.3 \pm 0.6	64.7 \pm 3.5	68.3 \pm 1.2	47.0 \pm 8.0	58.3 \pm 7.5	81.0 \pm 1.7
1A2	8.7 \pm 2.5	NS	31.3 \pm 5.1	NS	NS	12.0 \pm 2.6	12.3 \pm 4.5	38.3 \pm 7.5	29 \pm 11.5
2A1	NS ^b	11.3 \pm 3.5	NS	15.3 \pm 3.5	NS	22.6 \pm 2.5	NS	NS	20.3 \pm 2.9
2B1	14.3 \pm 4.7	NS	NS	NS	NS	13.0 \pm 2.6	NS	NS	NS
2C6	88.7 \pm 2.1	95.0 \pm 1.0	97.7 \pm 2.3	50.0 \pm 2.6	39.0 \pm 2.6	55.7 \pm 2.9	83.7 \pm 5.0	88.7 \pm 2.1	91 \pm 1.7
2C11	68.7 \pm 5.8	97.3 \pm 0.6	94.3 \pm 2.5	NS	58.3 \pm 7.5	79.7 \pm 4.2	77.7 \pm 15.9	82.0 \pm 5.3	89.3 \pm 0.6
2C12	12.7 \pm 3.2	NS	NS	14.7 \pm 1.5	NS	NS	NS	NS	NS
2C13	NS	NS	NS	NS	NS	NS	NS	NS	12.7 \pm 0.6
2D1	NS	NS	NS	15.7 \pm 5.1	42.3 \pm 1.5	12.3 \pm 4.6	NS	NS	NS
2D2	NS	NS	4.0 \pm 1.0	NS	NS	11.0 \pm 4.4	NS	NS	11.0 \pm 2.0
3A1	40.7 \pm 8.3	81.7 \pm 0.6	45.0 \pm 7.0	46.3 \pm 10.4	70.7 \pm 5.9	59.7 \pm 9.3	30.3 \pm 5.5	NS	51.3 \pm 1.5
3A2	30.3 \pm 4.5	56.3 \pm 3.8	33.0 \pm 9.0	24.3 \pm 2.5	38.3 \pm 3.5	26.7 \pm 7.2	16.7 \pm 1.2	13.3 \pm 3.5	29.7 \pm 5.9

^a% parent pyrethroid metabolized during a 10 min incubation; mean \pm SD, N=3

^bThe percent metabolism of pyrethroids by rat P450s was assessed by a one sample t-test. Values that were not significantly different from zero were labeled NS (p<0.05).

Table 8 % Metabolism of Type I and Type II pyrethroids by human P450 isoforms

Isoform	Bifenthrin	S-bioallethrin	Bioresmethrin	β - Cyfluthrin	λ -Cyhalothrin	Cypermethrin	cis- Permethrin	trans- Permethrin	Resmethrin
1A1	NS ^a	NS	NS	11.7 \pm 3.2	15.0 \pm 4.4	NS	13.3 \pm 3.8	27.0 \pm 2.6	NS
1A2	NS	NS	53.7 \pm 2.5	24.0 \pm 4.6	17.7 \pm 4.0	22.0 \pm 2.6	22.0 \pm 2.6	24.3 \pm 2.1	NS
2B6	NS	NS	32.7 \pm 5.0	NS	10.7 \pm 1.5	NS	NS	NS	NS
2C8	14.3 \pm 0.6 ^b	22.0 \pm 1.0	29.7 \pm 2.5	49.7 \pm 3.2	15.7 \pm 2.1	34.7 \pm 2.3	19.3 \pm 2.3	18.3 \pm 2.5	24.3 \pm 1.5
2C9*1	23.3 \pm 4.2	NS	32.3 \pm 6.4	26.0 \pm 5.3	18.0 \pm 6.1	NS	39.3 \pm 2.9	NS	37.0 \pm 7.0
2C9*2	6.3 \pm 6.0	15.0 \pm 3.0	28.0 \pm 10.5	NS	NS	12.3 \pm 4.5	NS	22.6 \pm 2.1	27.0 \pm 7.2
2C9*3	4.3 \pm 5.9	NS	NS	NS	8.7 \pm 2.5	16.7 \pm 3.1	NS	NS	NS
2C19	97.7 \pm 0.6	90.7 \pm 1.2	88.7 \pm 4.0	82.7 \pm 2.5	60.7 \pm 1.2	67.7 \pm 2.1	83.3 \pm 4.5	89.7 \pm 0.6	89.0 \pm 1.0
3A4	NS	13.0 \pm 3.5	15.7 \pm 1.2	21.7 \pm 3.8	31.7 \pm 5.5	22.0 \pm 4.4	25.0 \pm 9.6	NS	22.0 \pm 1.7

^aThe percent metabolism of pyrethroids by human P450s was assessed by a one sample t-test. Values that were not significantly different from zero were labeled NS (p<0.05).

^b% parent pyrethroid metabolized during a 10 min incubation; mean \pm SD, N=3.

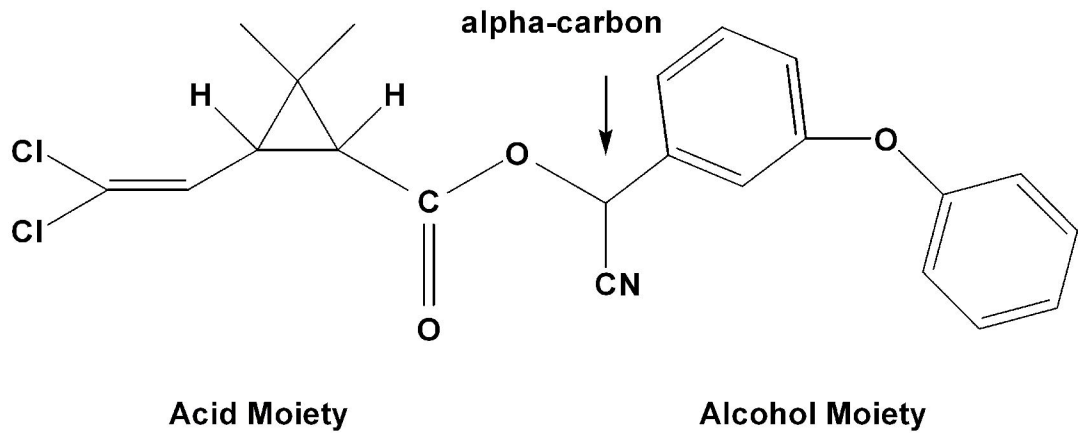
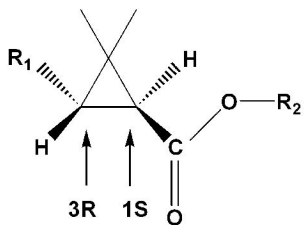
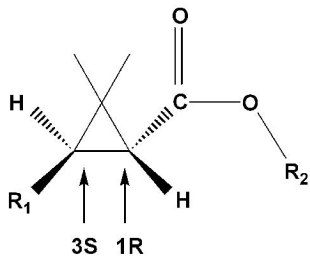


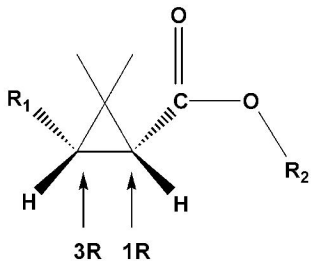
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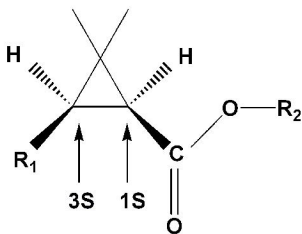
1S, 3R-trans enantiomer



1R, 3S-trans enantiomer

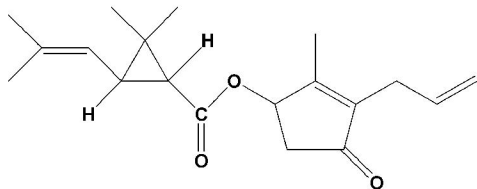


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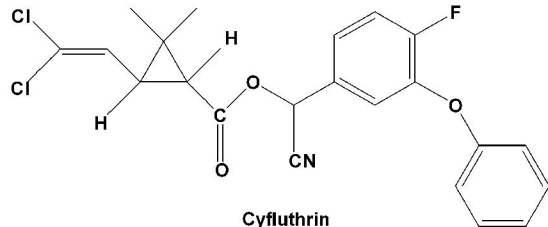


1S, 3S-cis enantiomer

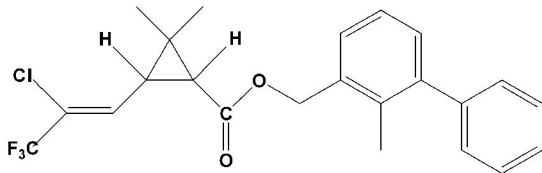
Figure 2



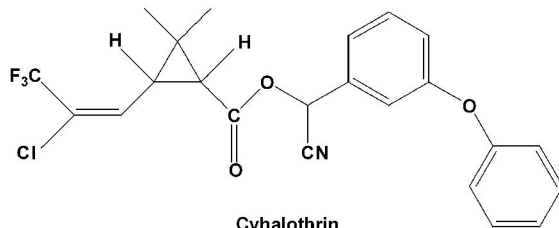
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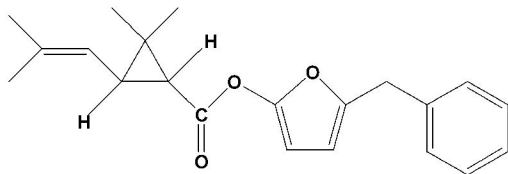
Cyfluthrin



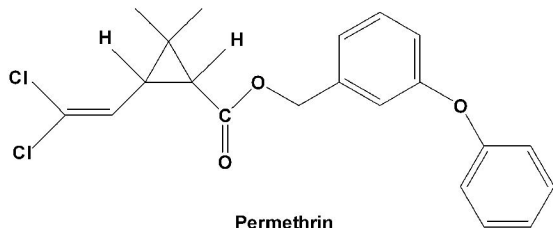
Bifenthrin



Cyhalothrin



Bioresmethrin



Permethrin

Figure 3