Parameters for Pyrethroid Insecticide QSAR and PBPK/PD Models for Human Risk Assessment

(March 13, 2012)

James B. Knaak*, Curtis C. Dary, Xiaofei Zhang, Robert W. Gerlach, R. Tornero-Velez, Daniel T. Chang, Rocky Goldsmith and Jerry N. Blancato

J.B. Knaak* (Corresponding author: jbknaak@aol.com)

Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, SUNY at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA.

C.C. Dary

U.S. Environmental Protection Agency, Human Exposure and Atmospheric Sciences Division, 944 East Harmon Avenue, Las Vegas, NV 89193-3478, USA.

X. Zhang · R.W. Gerlach

General Dynamics Information Technology, 181 North Arroyo Grande Boulevard, Suite 105A, Henderson, NV 89074-1624, USA.

R. Tornero-Velez · D. Chang · R. Goldsmith, Mail Code E205-01;

J.N. Blancato, Mail Code B143-04

U.S. Environmental Protection Agency, National Exposure Research Laboratory, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA.

Contents

1 Introduction

This pyrethroid insecticide parameter review is an extension of our interest in developing quantitative structure activity relationship-physiologically-based pharmacokinetic/pharmacodynamic (QSAR-PBPK/PD) models for assessing health risks, which interest started with the organophosphorus (OP) and carbamate insecticides (Knaak et al. 2004, 2008). The parameters shown in Table 1 (Blancato et al. 2000) are needed for developing pyrethroid PBPK/PD models, as is information on the metabolic pathways of specific pyrethroids in laboratory test animals and humans. Parameters may be obtained by fitting the output from models to experimental data gathered from in vivo studies (Zhang et al. 2007; Nong et al. 2008), in conjunction with using (1) experimental data obtained from in vitro studies, (2) quantitative structure-activity relationships (QSAR) and (3) other mathematical models, such as the mechanistic Poulin-Theil (2000, 2002a,b) algorithms for obtaining blood:tissue partition coefficients.

[Insert Table 1 about here.]

In this review, we have concentrated on the development of (1) in vivo metabolic data (i.e., V_{max} and K_m, etc.), (2) QSAR, and (3) mechanistic models and their application for building PBPK/PD models. The development of the pyrethroid insecticides for agricultural and home use is complicated by their chemistry, in that they each possess one to four chiral centers, increasing the number of isomeric forms by a factor of 2^n (where n = the number of chiral centers). Isomer mixtures and individual isomers are commonly both subjected to testing for insecticidal activity. The fewer the number of active forms,

the easier it is to test them for insecticidal activity, toxicity, and to build PBPK/PD models for them. The pyrethroids on which we focus in this review are presented in Table 2, along with their trivial and CAS names and their structures. Table A1 (Appendix A) defines the acronyms and abbreviations used in the text, while Table A2 (Appendix A) defines the chemical and mathematical expressions that are presented in this review.

[Insert Table 2 about here.]

2 Nature of Pyrethrin and Pyrethroid Insecticides

LaForge and Haller (1936) was first to elucidate the structure of natural pyrethrum, which they demonstrated constituted esters of chrysanthemic acid (CA). The natural pyrethrins (I and II), depicted in Figs. 1 and 2, are structures comprised of cyclopropanecarboxylic acids esterified with alkenylmethyl cyclopentenolones. All natural pyrethrins contain the cyclopropanecarboxylic acid moiety that is substituted in various ways at the 3'-position of the cyclopropane ring (A position) and at the carboxylic acid (B position). Unlike the substituent at the A position (C-3 of cyclopropane ring), the gem-dimethyl at position C-2 of the ring is critical to insect toxicity. Pyrethrin I/II and allethrin (Figs. 1, 2, Table 2, 2.1), each contain a chiral carbon at the CH or 7'-position of the cyclopentenolone ring.

[Insert Figure 1 about here.]

[Insert Figure 2 about here.]

2.1 Discovery

The synthetic era for the pyrethroids began with the replacement of the cyclopentenolone groups with other alcohol moieties, which resulted in the production of several household use products, and this success was followed by replacing the cyclopropane ring with a number of other acid functional groups. The cyclopropane ring changes were intended to produce pyrethroids beneficial for outdoor and agricultural insect control. The last changes to be made involved modifying the ester linkages (e.g., ethers, oximes, alkanes and alkenes) (Katsuda 1999). According to Nishizawa (1971), the natural pyrethrin compounds are optically active, whereas the synthetic pyrethroids used in the early studies were optically inactive. Optically active pyrethroids prepared from (+)-*trans*-chrysanthemic acid were superior to those prepared from optically inactive (±)-*cis*-, *trans*- compounds. Racemization and isomerization were used to convert the (-)-*trans* and (±)-*cis* isomers into the desired (+)-*trans* isomer.

The change in the alcohol moiety to allethrone led to the development of the first synthetic pyrethroid, allethrin. It also led to improved chemical stability of the natural pyrethrins and to reduced cost, because the original pyrethrins had been sourced from natural products (LaForge and Soloway 1947). The stability of allethrin made it superior to the natural pyrethrins in both kill and knock-down effects against mosquitoes.

Allethrin's successful discovery was followed by the development of other successful pyrethroids, to wit, tetramethrin (Kato et al. 1965) (1965, patent appl date) from tetrahydrophtalimide, resmethrin (Elliott et al. 1967) (1967, patent appl date) from 5-benzyl-3-furylmethyl alcohol and phenothrin (Fujimoto et al. 1973) (1968, patent appl date) by changing α-benzylfuran to phenoxyphenyl.

Additional changes to the acid moiety led to the development of fenpropathrin (Matsuo et al. 1976) (1971, patent appl date), permethrin, cypermethrin and deltamethrin (Elliott et al. 1974) (1972, patent appl date), fenvalerate (Ohno et al. 1974) (1973, patent appl date), fluvalinate (Henrick 1977) (1975, 1977 patent appl dates; Katsuda 1975), tralomethrin (Martel 1976) (1976, patent appl date) and cyhalothrin (Huff 1978) (1977, patent appl date). Unfortunately, patent application dates were unavailable for cyfluthrin, tefluthrin or bifenthrin (Katsuda 1999).

Resmethrin was the first synthetic pyrethroid insecticide that possessed insecticidal activity approaching the potency of pyrethrin I. In resmethrin, the furan ring replaced the cyclopentenone ring of pyrethrin I, and the benzene ring replaced the diene side chain of pyrethrin I. The success of resmethrin (four isomers, two *cis*- and two *trans*-) led to the synthesis of a form that was less toxic to mammals, bioresmethrin (1R-*trans* isomer). Unfortunately, bioresmethrin *per se* was no more photostable than were the earlier synthetic pyrethroids, because both the furan ring and isobutenyl side chain were sites vulnerable to degradation.

The bioresmethrin work led to the development of biopermethrin (new alcohol side chain and dihalovinyl group), and later to the introduction of a α-cyano group to yield cypermethrin. Insect activity is the greatest when the side chain is *cis* to the ester group. Deltamethrin was discovered by replacing a bromine for a chlorine atom in the acid side chain, making it possible to isolate a single crystalline product ([1R, *cis*] α-S isomer) with all three centers resolved (Elliott et al. 1974). Permethrin, cypermethrin and deltamethrin were introduced by Elliott et al. (1974, 1978), Elliott and Janes (1978), Elliott (1989), and Fisher et al. (1983). Phenothrin contains four optical and stereo isomers, with only the [1R, *trans*] and [1R, *cis*) isomers being insecticidally active. Metabolism studies were carried out with these individual isomers (Izumi et al. 1984; Kaneko et al. 1981a, 1984; Miyamoto et al. 1974; Suzuki et al. 1976). The discovery of fenpropathrin, with its relatively simple structure, was first announced in 1973 by Sumitomo Chemical Co., and was commercially introduced as an acaricide in 1980. Sumitomo Chemical Co. introduced esters of the substituted phenyl acetic acids in 1974, replacing the cyclopropane pyrethroids. This led to the first commercial pyrethroid that was photostable, viz., fenvalerate. The racemic mixture of this product was followed in 1986 by the single (S, S) isomer, esfenvalerate.

The development of fenvalerate and later esfenvalerate led to the synthesis and commercial marketing of fluvalinate, which is active against spider mites (Henrick et al. 1980). Fuchs synthesized all of the seven possible fluoro substituted isomers of cypermethrin (e.g., 2, 4, 5, 6, 2', 3' and 4') (Hamman and Fuchs 1981). Substitution at the 4'-position produced the most active compound, cyfluthrin. Activity was almost

eliminated when chlorine was substituted for fluorine in the 4'-position. Tefluthrin has a higher vapor pressure than most of the other pyrethroids and is active against soil pests such as the corn root worm (Jutsum et al. 1986). Bifenthrin is an ester with alcoholic components whose activity is not enhanced by an alpha-cyano substituent. Bifenthrin is active against insects (aphids) and mites (Plummer et al. 1983).

2.2 Isomers and Technical Products

The pyrethroids addressed in this review are esters of chiral acids (e.g., dimethylcyclopropane-carboxylic acid, chrysanthemic acid) and alcohols (e.g., (S) cyclopentenolones, 3-phenoxybenzyl, α-cyano-3-phenoxybenzyl). These pyrethroids possess n = one to four chiral centers resulting in 2^n isomers per pyrethroid. The pesticidal activity of each isomer varies, with only one or two of the isomers being active against insect pests. The stereochemistry and structure of these isomers may be mentioned in the literature when they were subjected to biological study. However, a complete listing of the isomers by chemical name or by CAS numbers rarely appears in either published biological studies, or in analytical studies involving chromatographic separations. Terms such as enantiomers and diastereoisomers and their definitions are often used in conjunction with studies involving these isomers, particularly in the case of analytical, biological or toxicological studies. At best many of these terms are confusing to the reader and the use of some of the terms in the literature may be incorrect.

From one to all possible isomers exist in technical pyrethroid products sold by manufacturers of these pyrethroids, and their combined content is given as a percentage. Technical products are used by companies to formulate finished products that are used in and around homes and gardens. Regulators require the development of appropriate analytical methods to identify both registered “actives” and the "toxic" metabolites that appear in urine and tissues or in edible crops. However, it is common for multiple different chromatographic methods to be used for the analysis of pesticide mixtures (Eadsforth and Baldwin 1983). For pyrethroids, methods adequate to trace the *cis* vs. *trans* isomer content are important, and depend on the nature of the registered “active” or technical products and also on the nature of their biotransformation products.

In Appendix B, Tables B1-B30, we provide depictions of and CAS numbers for the chiral isomers for each of the 15 pyrethroids addressed in this review. The stereoisomer codes (A, B, C, etc.) were used in these tables to specify chiral configurations (i.e., (1R, 3S) (α S)), optical rotation, *cis*/*trans* and enantiomers pairs, and CAS no. The Chemical Abstracts Index Guide, (Appendix IV 2002) provides a discussion of the stereochemical descriptors found in the CAS index names. The CAS rules are consistent with IUPAC recommendations and produce readily interpreted stereochemical names. R and S are employed for chiral elements possessing either absolute or relative stereochemistry. E and Z are used primarily to describe geometrical isomerism about double bonds. The relative terms *cis*, *trans*; α and β are used as alternatives to R and S. The absolute terms R and S are based on the Cahn-Ingold-Prelog Sequence rule (Cahn et al. 1966) and depend on the ranking of atoms or groups attached to the stereochemical element or chiral atom.

R is assigned to a clockwise sequence, whereas S denotes a counterclockwise sequence of atoms represented by a, b, c, and d about the chiral center. For example, 3R of pyrethrin I (Fig. 1) is represented by the rule in Fig. 3,

[Insert Figure 3 about here.]

wherein the least preferred atom or group 'H' is considered to be below the plane of the paper, while the other groups project outward toward the viewer. The structure for pyrethrin I (Fig. 1, 1R, 3R) indicates that 1R is *trans* to the 3-position as shown in Fig. 4.

[Insert Figure 4 about here.]

Where they exist, we also identify enantiomeric pairs. A molecule with n stereogenic centers has 2^n stereoisomers (except when special symmetry conditions are present). When two stereoisomers are mirror images of each other they form an enantiomer pair. Each stereoisomer has only one enantiomer. Any other pairing of stereoisomers is considered to be a diastereomer pair. Thus, when there are 3-stereogenic centers, one has 8 stereoisomers that can be arranged into 4 enantiomer pairs and 24 diastereomer pairs. For the above example that has eight stereoisomers, each stereoisomer is associated with one enantiomer and six diastereomers. Each stereoisomer is simultaneously an enantiomer and a diastereomer, unless the discussion is limited to just one pair of stereoisomers. The literature becomes difficult to understand and interpret when discussions of stereoisomers blur the above definitions and are unclear as to which stereoisomer or stereoisomer pair is being reported on. One should not report on a

diastereomer unless its paired stereoisomer is also identified, and enantiomers should only be discussed as a pair of compounds.

A geometric isomer contribution from the presence of double bonds does not qualify as a stereocenter. When double bonds affect stereochemistry, the enantiomers are due only to chiral differences associated with a stereocenter. The enantiomers are always paired with identical configurations about the double bond. Both isomers must be cis- or trans- to meet the mirror image requirement that defines an enantiomer pair.

2.3 Stereochemistry

The following paragraphs describe the stereochemistry of the 15 pyrethroids addressed in this review.

Allethrin: Table B1, Appendix B, gives the chiral configurations of the 8 individual isomers of allethrin, their optical rotation, *cis*/*trans* configurations, CAS number for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A through H making it easier to identify (label) each isomer. In Table B2, Appendix B, the common and proprietary names used by registrants for technical products are presented. Each product contains more than one isomer, except for dl-*trans*-allethrin, which is 100% "H" isomer. The "B" isomer is the most pesticidal isomer of the eight allethrin isomers.

Bifenthrin: Table B3, Appendix B, gives the chiral configurations of the 4 individual isomers of bifenthrin having a Z-configuration for the double bond, their optical rotation, *cis*/*trans* configurations, and CAS no. for absolute stereochemistry. The structure of each stereoisomer is coded A through D making it easier to identify (label) each isomer. In Table B4, Appendix B, the common name of one technical product sold by registrants is listed. The Technical product contains isomers A and D, comprising 100% of the active ingredients.

Cyfluthrin: Table B5, Appendix B, gives the chiral configurations of the 8 individual isomers of cyfluthrin, their optical rotation, *cis*/*trans* configurations, CAS no. for absolute stereochemistry and the 4 enantiomer pairs (mirror images). The structure of each stereoisomer is coded A through H making it easier to identify (label) each isomer. In Table B6, Appendix B, the common name of two technical products sold by registrants is listed. Cyfluthrin is a mixture of all eight isomers. The *cis*/*trans* isomers (B and G, D and E) comprise up to 95% of the active ingredients in beta-cyfluthrin.

Cyhalothrin: Table B7, Appendix B, gives the chiral configurations of 8 individual isomers of cyhalothrin having a Z-configuration about the double bond, their optical rotation, *cis*/*trans* configurations, and CAS no. for absolute stereochemistry. No enantiomer pairs are shown. The structure of each stereoisomer is coded A through H making it easier to identify (label) each isomer. In Table B8, Appendix B, the common name of two technical products sold by registrants is listed. Cyhalothrin is a mixture of four *cis* isomers (A, B, G and H), while the most used technical product, lambda

cyhalothrin, is a mixture of isomers B and G. Gamma cyhalothrin contains only one isomer, the 'B' isomer.

Cypermethrin: Table B9, Appendix B, gives the chiral configurations of the 8 individual isomers of cypermethrin, their optical rotation, *cis/trans* configurations, CAS no. for absolute stereochemistry and the 4 enantiomer pairs (mirror images). The structure of each stereoisomer is coded A through H making it easier to identify (label) each isomer. In Table B10, Appendix B, the common name of five technical products sold by registrants is listed. Cypermethrin is a mixture of all eight *cis/trans* isomers (A-H); however, the most used technical product, α-cypermethrin, is a mixture of the *cis* isomers B and G. Theta-cypermethrin contains the two *trans* isomers, D and E, whereas β-cypermethrin is a 2:3 mixture of α-, and θ-cypermethrin.

Deltamethrin: Table B11, Appendix B, gives the chiral configurations of the 8 individual isomers of deltamethrin, their optical rotation, *cis*/*trans* configurations, the CAS no. for absolute stereochemistry and enantiomer pairings. The structure of each stereoisomer is coded A through H making it easier to identify (label) each isomer. In Table B12, Appendix B, deltamethrin is shown as the name for the technical product containing only stereoisomer B. Small quantities of the other isomers are most likely present in technical deltamethrin.

Esfenvalerate/fenvalerate: Table B13, Appendix B, gives the chiral configurations of the 4 individual isomers of fenvalerate, their optical rotation, *cis*/*trans* configurations, CAS

no. for absolute stereochemistry and the 2 enantiomer pairs (mirror images). The structure of each stereoisomer is coded A through D making it easier to identify (label) each isomer. In Table B14, Appendix B, esfenvalerate and fenvalerate (Pydrin) are listed as the two technical products sold by registrants. These two technical products contain all four isomers, with esfenvalerate having isomer 'D' as the major component. Fenvalerate (pydrin) contains equal amounts of each of the four isomers.

Fenpropathrin: Table B15, Appendix B, gives the chiral configurations of the 2 individual isomers of fenpropathrin, their optical rotation, and CAS no. for absolute stereochemistry. The structure of each stereoisomer is coded A and B making it easier to identify (label) each isomer. In Table B16, Appendix B, fenpropathrin is identified as the only technical product sold by registrants. This technical product contains both isomers.

Fluvalinate: Table B17, Appendix B, gives the chiral configurations of the 4 individual isomers of fluvalinate, their optical rotation, CAS no. for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A-D making it easier to identify (label) each isomer. In Table B18, Appendix B, fluvalinate and *tau*-fluvalinate are listed as the only technical products sold by registrants. Technical fluvalinate contains all four isomers, while *tau*-fluvalinate is limited to isomers 'A' and 'B'.

Permethrin: Table B19, Appendix B, gives the chiral configurations of the 4 individual isomers of permethrin, their optical rotation, CAS no. for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A-D making it easier to

identify (label) each isomer. Table B20, Appendix B, lists permethrin, *cis*-permethrin, *trans*-permethrin and biopermethrin as technical products sold by registrants. Technical permethrin contains all four isomers, while *cis*/*trans* permethrin contains isomers A, D and B, C, respectively. Biopermethrin contains only isomer 'B'.

Phenothrin: Table B21, Appendix B**,** gives the chiral configurations of the 4 individual isomers of phenothrin, their optical rotation, CAS no. for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A-D making it easier to identify (label) each isomer. In Table B22, Appendix B, phenothrin, *cis*-phenothrin, *trans*-phenothrin, and sumithrin are listed as technical products sold by registrants. Technical sumithrin contains one isomer (A), while *trans*/*cis* phenothrin contain isomers A, D and B, C, respectively.

Resmethrin: Table B23, Appendix B, gives the chiral configurations of the 4 individual isomers of resmethrin, their optical rotation, CAS no. for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A-D making it easier to identify (label) each isomer. In Table B24, Appendix B, resmethrin, *cis*-resmethrin, *trans*-resmethrin, bioresmethrin and cismethrin are listed as technical products sold by registrants. Technical bioresmethrin contains one isomer (A), while *trans*/*cis* phenothrin contains isomers A, D and B, C, respectively.

Tefluthrin: Table B25, Appendix B, gives the chiral configurations of the 4 individual isomers of tefluthrin having 'Z' configuration at the chiral double bond, their optical

rotation, and CAS no. for absolute stereochemistry. The structure of each stereoisomer is coded A-D making it easier to identify (label) each isomer. In Table B26, Appendix B, tefluthrin is identified as the only technical product sold by registrants. Technical tefluthrin contains two *cis*-isomers (A, D). Isomer A is the main active component.

Tetramethrin: Table B27, Appendix B, gives the chiral configurations of the 4 individual isomers of tetramethrin, their optical rotation, CAS no. for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A-D making it easier to identify (label) each isomer. In Table B28, Appendix B, tetramethrin, D-tetramethrin and (+)-*cis*-tetramethrin are identified as the technical products sold by registrants. Technical (+)-*cis*-tetramethrin contains one *cis* isomer (B). D-tetramethrin is composed of isomers A and B in a 4:1 ratio.

Tralomethrin: Table B29, Appendix B, gives the chiral configurations of the 16 individual isomers of tralomethrin, their optical rotation, CAS no. for absolute stereochemistry, and enantiomer pairs. The structure of each stereoisomer is coded A-P making it easier to identify (label) each isomer. In Table B30, Appendix B, tralomethrin (isomers G and H) is listed as the only technical product sold by registrants.

2.4 Analytical Methods for Pyrethroid Isomers

The U.S. EPA requires registrants of pesticides to provide suitable analytical methods to affirm the identity of the pesticides they test. Such methods are needed for: acute and

chronic toxicology studies, special studies (i.e., metabolism, pharmacokinetic, human exposure, animal residues, etc.), product and environmental chemistry studies, efficacy, health effects testing (i.e., functional observation battery, peripheral nerve function) and for pesticide residue enforcement efforts. In the past, registrants provided gas chromatographic methods for each active ingredient (i.e., permethrin, cypermethrin, etc.), with all the isomers chromatographing as a single component. Gas chromatography (GC), performed with open tube or packed columns containing polar (carbowax) or nonpolar (silicones, polysiloxane) phases, was the primarily instrument used in the 1950s-1960s to analyze environmental samples for pesticide residues, including pyrethroids such as permethrin. The GC columns then available did not separate the stereoisomers present, because their partition coefficients are so similar. Low pressure columns packed with alumina or silica gel were often used to clean up samples prior to gas chromatography, but were not routinely used for chromatographic analysis of pesticide residues. Thin-layer chromatographic (TLC) plates (e.g., silica gel 60 F254) were used by Kaneko et al. (1981a,b,c, 1984); Ruzo and Casida (1977), and Ruzo et al. (1978, 1979) for separating and identifying radiolabeled parent pyrethroids and their metabolites. The resolution achieved on these plates, by using two-dimensional chromatography and up to 7 solvent systems (Kaneko et al. 1981a,b,c, 1984), was quite remarkable considering the nonpolar and polar nature of the products being separated.

The introduction of capillary gas chromatographic columns (30 meter x 0.53 mm) in about 1970 that were run in split and splitless modes provided a means for separating greater numbers of pesticides. However, analyses by gas chromatography, in which only conventional achiral stationary phases in capillary columns are used, are not capable of

separating the pyrethroid isomers. Gas chromatographic analysis of the pyrethroid isomers is best achieved by hydrolysis of the pyrethroid, methylation of the acids or alcohols, followed by GC chromatographic analysis.

Because of the problems encountered in separating the pyrethroid isomers by gas chromatography, chiral specific (CSP) HPLC (High Pressure Liquid Chromatography) columns were developed. These utilized a base support such as silica along with large groups such as (R)-phenylglycine and 3, 5-dinitrobenzoic acid-amide linkages attached to Si groups. These groups are varied so that they can be matched to the nature of the analytes being separated.

The chiral columns used for liquid chromatography may also be used for supercritical fluid chromatography (SFC). SFC offers important advantages over HPLC and GC in the separation of enantiomers. First, SFC provides a higher resolution per unit of time than does LC, because the diffusion rates in the mobile phase and linear velocities are higher. Second, SFC chromatography is carried out at temperatures well below those used in GC. LC and GC detectors, such as FID (flame ionized detectors) and mass spectrometry (MS), may also be applied to SFC (Chamberlain et al. 1998).

A search of the literature involving application of chiral chromatographic columns for separating pyrethroid isomers turned up the information that is summarized in

Appendix C, Tables C1-C15. This information (i.e., chiral columns, isomers chromatographed, solvent systems and results) includes recent work performed on the

separation of the isomers of the 15 pyrethroids we address in this review. A brief history of this work is reviewed in the following sections for each of the pyrethroids.

Allethrin: D-allethrin has 8 isomers ($2^3 = 8$), 4 *cis* (C, D, E, and F) and 4 *trans* (A, B, G, H) isomers (Table C1, Appendix C). Mancini et al. (2004) separated *cis/trans* isomers from each other on an achiral silica HPLC column using n-hexane:tert-butyl methyl ether (96:4) (v/v) as the mobile phase. The *trans* isomers were separated (G, H, A, B, respectively) from each other on a CHIRAL-CEL OJ using n-hexane-*tert*-butyl methyl ether (90:10) (v/v). This same column was used to separate the *cis* isomers (F, D, C, and E, respectively) using n-hexane:isopropanol (99.3:0.7) (v/v). Kutter and Class (1992) were able to separate the *trans* allethrin isomers on a chiral β-cyclodextrin RP-HPLC column, but were unable to separate the *cis* isomers.

Bifenthrin: Bifenthrin has 4 isomers ($2^2 = 4$) with the Z configuration about the double bond (Table C2, Appendix C). Technical bifenthrin is comprised of two *cis* isomers, A and D (Table B3, Appendix B). Liu et al. (2005a) separated the two isomers on a β-cyclodextrin-coated BGB-172 gas chromatographic column with 'A' chromatographing first (54.3 min) followed by 'D' (55.5 min). Chromatography on a Sumichiral OA-2500-1 HPLC column using n-hexane, isopropanol and ethanol (99.9/0.6/0.14) reduced the retention time down to 10.57 min for 'A' and 11.25 min for 'D' (Liu et al. 2005b).

Cyfluthrin: Cyfluthrin has 8 isomers ($2^3 = 8$) (Table C3, Appendix C). Liu et al. (2005b) separated all 8 stereoisomers in the order A, H, G, B, C, F, E and D using a Chirex 00G-3019-DO HPLC column (Phenomenex; Torrance, CA). The mobile phase consisted of n-hexane:1, 2-dichloroethane:ethanol (500:10:0.05, v/v/v). Li et al. (2003) separated 4

stereoisomers (enantiomer pairs II and IV) on a Chiralcel OD HPLC column; mobile phase n-hexane:2-propanol (100+2, v/v). All four isomers were baseline separated, with *cis* isomers eluting prior to *trans* isomers. Faraoni et al. (2004) used a dual-column approach to successfully separate all eight stereoisomers. Enantiomer pairs were separated using a CNP silanized silica gel HPLC column with n-hexane:2-propanol, (99.0:0.1, v/v). One sample split was analyzed with a Chiralcel OD-H HPLC column, n-hexane:2-propanol (99.4:0.5) (v/v), giving peak separation for stereoisomers A, G, H, B, E, and D, in that order. A second split was routed through a Pirkle-type CSP (DNBPG) HPLC column (Aldrich-Chimica, Milan, IT) with n-hexane:2-propanol (99.9:0.1, v/v), separating peaks C and F.

Cyhalothrin: Cyhalothrin has 8 isomers ($2^3 = 8$) (Table C4, Appendix C). The *cis* isomers are generally more toxic than the corresponding *trans* isomers. In practice, cyhalothrin is produced only in the Z and *cis* forms, reducing the number of isomers to four. These comprise two pairs of isomers: pair A and H: (Z), (1R, 3R), R-α-cyano and (Z), (1S, 3S) S-α-cyano and pair B and G: (Z), (1R, 3R), S-α-cyano and (Z), (1S, 3S) R-α-cyano. Pure γ-cyhalothrin is a racemic mixture of isomers B and G. A normal phase Nucleosil Sherisorb CN column (Shimadzu, Kyoto, Japan) was used to separate this mixture. The mobile phase was n-hexane:tetrahydrofuran:2-propanol (99:0.9:0.1, v/v/v) (Rao et al. 2004). The isomers chromatographed in the following order: B, G, H and A. The major isomers in λ-cyhalothrin were B and G with small quantities of A and H. Yang, G-S et al. (2004) separated the principal isomers (B and G) of commercially produced λ-cyhalothrin on a CHIRALCEL OD-R column using acetonitrile:water (30:70, v/v). The elution order was not indicated by the authors.

CHIRALCEL OD and three other chiral columns CHIRALCEL (OJ), CHIRALPAK (AD) and CHIRALPAK (AS) were used to chromatograph and separate the two isomers of λ-cyhalothrin, B + G (Xu et al. 2007). The order (B or G first) in which they chromatographed was not indicated by the authors.

Cypermethrin: Cypermethrin has 8 isomers ($2^3 = 8$) (Table C5, Appendix C). Liu et al. (2005b) and Tan et al. (2007) used two Chirex OOG-3019-OD columns to separate the eight isomers in the following order: A, H, G, B, C, F, E, and D. *Cis* isomers chromatographed before the *trans* isomers. Tan et al. (2007) used n-hexane:1, 2-dichloromethane:2-propanol (96.8:3:0.2, v/v/v) as the mobile phase, while Liu et al. (2005b) used n-hexane:1, 2-dichloroethane:ethanol (500:30:0.15, v/v/v) to separate the isomers. A CHIRALCEL OD column was used by Li et al. (2003) to separate the (1:1 *cis*/*trans*) isomers in β-cypermethrin. The solvent system consisting of n-hexane:2-propanol (100 + 2, v/v) separated β-cypermethrin into four peaks, B (*cis*), D (*trans*), G (*cis*) and E (*trans*), in their respective order. This order was unusual, based on the studies of Liu et al. (2005b) and Tan et al. (2007), wherein the *cis* isomers chromatographed before the *trans* isomers. Wang et al. (2004) prepared a chiral stationary phase by bonding cellulose-tris (3, 5-dimethylphenyl-carbamate) on aminopropylsilica that separated seven of eight stereoisomers in less than 25 min. A Partisil silica column separated the eight isomers of cypermethrin into four enantiomer pairs (A + H; B + G; C + F; D + E). The mobile phase was n-hexane:diethyl ether (500:10, v/v) (Wang et al. 2004).

Deltamethrin: Deltamethrin has 8 isomers ($2^3 = 8$) (Table C6, Appendix C). According to Cayley and Simpson (1986), a cyano-bonded column was capable of chromatographic separation of deltamethrin (isomer B) from isomer A, by using 0.1% 2-propanol in n-hexane as the mobile phase. Technical deltamethrin contains only one isomer, "B". Yang, G-S et al. (2004) used a Chiralpak AD HPLC column (GROM, Herrenberg-Kayh, Germany), with a mobile phase of ethanol:water (85:15, v/v), to separate isomer B from isomer G. A new analytical method for the determination of deltamethrin in TC, WP, EC, UL and DP was adopted by CIPAC (Collaborative International Pesticides Analytical Council), with provisional status, in 2004. This HPLC method used a cyano-column and detection at 230 nm (WHO, Deltamethrin 2005a). No chromatographic procedures were found that separated all seven isomers of deltamethrin from deltamethrin per se (isomer B).

Fenvalerate: Fenvalerate has 4 isomers ($2^2 = 4$) (Table C7, Appendix C). A CHIRALCEL OD column separated the enantiomer pair I (isomers A + D) from II (isomers B + C) (Li et al. 2006). In case of the four individual isomers, the first and second peaks were only partially resolved (Li et al. 2009). Huang et al. (1991) resolved fenvalerate into four well-separated peaks on a Pirkle-type 1-A chiral HPLC column using 99.9:0.1 hexane /isopropanol (v/v) as the mobile phase. The respective elution order was B, C, D and A. The enantiomer pair II eluted before pair I.

Esfenvalerate consists primarily of isomer D. Tan et al. (2007) reported on the complete separation of the four fenvalerate isomers by using a novel CSP (chiral specific phase) prepared by connecting (R)-1-phenyl-2-(4-methylphenyl)ethylamine (PTE) amide

derivative of (S)-isoleucine to aminopropyl silica gel, through a 2-amino-3,5-dinitro-1-carboxamido-benzene unit. The mobile phase was hexane 1, 2-dichloroethane 2-propanol (97.45:2.50:0.05). The order of elution was C, B, A and D, respectively. In an earlier study, Papadopoulou-Mourkidou (1985) used a chiral column (BAKERBOND) and a mobile phase of 0.1% methanol, 0.3% 2-propanol and 99.6% hexane (by volume) to separate all four isomers of fenvalerate.

Fenpropathrin: Fenpropathrin has 2 isomers ($2^1 = 2$) by virtue of possessing one chiral center (Table C8, Appendix C). Tan et al. (2007) reported the separation of the two isomers of fenpropathrin with a CSP column (that is described in the above paragraph as having also separated the four isomers of fenvalerate). The mobile phase was n-hexane 1, 2-dichloroethane 2-propanol (96.8:3.0:0.2). The 'A' isomer chromatographed prior to the 'B' isomer.

Fluvalinate: Fluvalinate has 2 enantiomer pairs and four isomers ($2^2 = 4$) (Table C9, Appendix C). Gao et al. (1998) separated the enantiomers pairs (I, A + D; II, B + C) on a Pirkle-type chiral phase HPLC column. Yang G-S et al. (2004) used a CHIRALCEL OJ column (250 x 2 mm ID) to separate the two isomers of tau-fluvalinate (A + B) with n-hexane ethanol (90:10, v/v) as the mobile phase. The CHIRALCEL OJ column was purchased from GROM (Herrenberg-Kayh, Germany).

Permethrin: Permethrin has 2 enantiomer pairs and 4 isomers ($2^2 = 4$) (Table C10, Appendix C). Liu HX et al. (2005) separated A + D (*cis* isomers) and B + C (*trans* isomers) on a Sumichiral OA-2500-I column by using n-hexane, isopropanol, and ethanol (99.9:0.6:0.14, v/v) as the mobile phase. The *cis* isomers chromatographed before the

trans isomers, in the order of A, D, B and C, respectively. A CHIRALCEL OJ column was used by Ulrich et al. (2008) to separate all four isomers of permethrin, with isopropanol and ethanol (2-5%) in n-hexane as the mobile phase. The isomers chromatographed in the order of D, A, B and C, respectively. The *cis* isomers (A + D) chromatographed before the *trans* isomers (B + C).

Phenothrin: Phenothrin has 2 enantiomer pairs and 4 isomers ($2^2 = 4$) (Table C11, Appendix C). Girelli et al. (2002) separated *cis*-1R-phenothrin (B) and *trans*-1R-phenothrin (A) on a CHIRALCEL OD-H column by using n-hexane:2-propanol (99.97:0.03, v/v). The (+) optical isomer (*cis*) chromatographed prior to the (-), or *trans* isomer. Separating these two isomers were also achieved by using a Chirex (S)-leu/(S)-NEA column or a 3, 5-DNB-α-phenylglycine column. The Chiralcel OD-H and Chirex (S)-leu / (S)-NEA columns were purchased from Daicel (Tokyo, Japan) and Chemtex Analytical (Bologna, Italy), respectively. The 3, 5-DNB-α-phenylglycine column was obtained by treating an Econosphere NH_2-5 column (Alltech, Italy) with 3,5-DNBPG ((-) (R)-N-(3,5-dinitrobenzoyl) α-phenylglycine). The four optical isomers of phenothrin were separated to the enantiomer level by using a covalently-bonded Pirkle-type 1-A HPLC column, and a mobile phase of 0.25 to 1% diethyl ether in hexane. Enantiomer separation was achieved by adding a second column that utilized a reverse phase Regis Pirkle-type 1-A ionic stationary phase (Deeside, UK), and n-hexane:2-propanol (99.975:0.025, v/v) mobile phase (Cayley and Simpson 1986).

Resmethrin: Resmethrin has 2 enantiomer pairs and 4 isomers ($2^2 = 4$) (Table C12, Appendix C). The columns employed by Girelli et al. (2002) to separate the *cis* and *trans*

1R-phenothrin isomers were also used to separate *cis*- from *trans*–resmethrin (enantiomer pairs: (B + C; A + D). The method used by Cayley and Simpson (1986) for phenothrin also separated the resmethrin isomers in the order B, C, A, and D. And, the method described by Oi et al. (1990) for phenothrin also separated all four resmethrin isomers.

Tefluthrin: Tefluthrin has 8 isomers ($2^3 = 8$), but only the Z-configuration is used in commercial products, giving 4 isomers of interest (Table C13, Appendix C). A search of the literature turned up no published studies that addressed the chromatographic separation of the isomers. Tefluthrin was developed by Syngenta Crop Protection and is based on the same Z-*cis*-acid that is used to produce λ-cyhalothrin. Tefluthrin is sold as a racemic mixture that contains equal amounts of the A and D isomers; the A isomer is the most active component.

Tetramethrin: Tetramethrin has 4 isomers ($2^2 = 4$) (Table C14, Appendix C). Base line separation of these four isomers was achieved by Zhe et al. (2008) by their use of a Chiralpak AD-H column (amylose 3,5-dimethylphenyl-carbamate) and a solvent system consisting of n-hexane:ethanol:2-propanol (99.0:0.9:0.1, v/v/v). The column was purchased from Daicel Chemical Industries Ltd., Japan. The isomers elute in the order of B, D, A and C, respectively. The *trans* isomers (D and A) yielded the largest peaks, because the sample of tetramethrin used was *trans*-isomer enriched. Zhe et al. (2008) used a UV detector in conjunction with a polarimeter to identify the isomers. According to Kurihara et al. (1997a,b), all of the 1R isomers give positive (+) peaks in the polarimeter.

Tralomethrin: Tralomethrin has 4 possible chiral centers (1C, 3C, αC, and CBr), giving rise to 16 possible isomers ($2^4 = 16$) (Table C15, Appendix C). The commercial product contains: G, (1R, 3S, αS, BrR) tralomethrin and H, (1R, 3S, αS, BrS) tralomethrin. Loss of bromide ion results in the formation of deltamethrin. A LiChroCART 125-4 Superspher 100 RP-18 column (Hewlett-Packard) was used by Valverde et al. (2001) to separate and detect the two diasteroisomers (G and H) of tralomethrin by LC-ES-MS.

2.5 Pyrethrins, Pyrethroids, and Their Chiral Components

In the natural pyrethrin esters, the presence of two asymmetric cyclopropane carbons implies the possible existence of four stereoisomers (Deltamethrin Monograph 1982). However, in the natural pyrethrins, only the (1R, 3R) configuration exists. This fact limits the number of acids (isomers) that need to be considered in developing analytical methods for detecting pyrethrin residues in food products, or animal tissues and fluids (i.e., blood, urine and feces). For the alcohol component, three alcohols exist: pyrethrolone, cinerolone and jasmolone, and all three possess an asymmetric center that has an (S) configuration.

The discovery of the synthetic pyrethroid insecticides presented chemists/biologists with the challenge of separating and identifying the isomers possessing the highest insecticidal activity. The esterification of allethrone with chrysanthemic acid, without resolution of the asymmetric centers, resulted in the formation of 2^3 (eight isomers) of which only the stereoisomers having the (S) configuration of allethrone were active insecticides. The

most active isomer, (S)-bioallethrin (1R, 3R, α-S) has the same asymmetric centers as does the pyrethrins, and is currently manufactured in large quantities.

The same synthetic approach (i.e., varying the acid, alcohol or both) resulted in the development of the commercially successful active pyrethroid insecticides covered in this review; this same approach also produced a host of relatively inactive isomers. The most potent constituent of resmethrin is the 1R, 3R isomer (bioresmethrin), and is formed during the esterification of (+) *cis*-, *trans*-chrysanthemic acid with 5-benzyl-3-furylmethyl alcohol to produce bioresmethrin and three other stereoisomers. Of greater interest to most chemists/biologists is the development of the eight isomers of deltamethrin, and specifically the active 1R, 3R, α-S isomer (Deltamethrin Monograph 1982), which was resolved from the isomer mixture by crystallization.

The lack of standardization, good analytical methods and a clear understanding of the chemistry of these pyrethroids has prevented interested parties from easily studying the details of the metabolism, pharmacokinetics, toxicity, and environmental fate of the individual isomers.

2.6 Isomers of Chrysanthemic Acids

In Table 3, we present the structures and names of the chrysanthemic acids that are used in the synthesis of allethrin, cyfluthrin, deltamethrin, permethrin, fenvalerate, phenothrin,

resmethrin and tetramethrin. These acids (isomers), depicted in Tables B31-B35, Appendix B, are released during metabolism and are excreted in the urine of exposed animals. A short discussion of each acid and their isomers is presented in the following paragraphs.

1. Chrysanthemic acid: Table B31, Appendix B, gives the 4 isomers, 2 *trans* and 2 *cis* isomers for chrysanthemic acid along with their optical rotation. These four isomers are the products of the hydrolysis of the allethrin, phenothrin and resmethrin.

2. Chrysanthemum dicarboxylic acid: In Table B32, Appendix B, the 4 isomers arising from the oxidation/hydrolysis of allethrin, fenpropathrin, phenothrin and resmethrin are listed. Oxidation of the parent molecule may occur prior to hydrolysis, or hydrolysis may occur followed by oxidation.

3. Permethrinic acid: Table B33, Appendix B, gives the 4 isomers arising from the hydrolysis of the 4 isomers of permethrin and the 8 isomers of cypermethrin and cyfluthrin.

4. Esfenvaleric acid/Fenvaleric acid: Table B34, Appendix B, gives the 2 isomers arising from the hydrolysis of esfenvalerate/fenvalerate.
5. Decamethrinic acid: Table B35, Appendix B, gives the four isomers arising from the hydrolysis of deltamethrin.

[Insert Table 3 about here.]

2.7 Analytical Chemistry, Acid Components

Chrysanthemic acid has n = 2 enantiomer pairs and 4 isomers ($2^n = 4$) (Table C16, Appendix C). Oi et al. (1995) and Oi (2005) readily separated these isomers on an HPLC CHIREX column Phase 3010 by using a mobile phase of 0.1M ammonium acetate in water/tetrahydrofuran (60:40). The four individual isomers of chrysanthemic acid are given in Table B31; Appendix B. Table 3 shows the generic structure of chrysanthemic acid. This moiety is the acid leaving group for allethrin, phenothrin, resmethrin and tetramethrin, which is further oxidized to E-*cis/trans* chrysanthemum dicarboxylic acid (Elflein et al. 2003). The isomers of the dicarboxylic acid are given in Table B32, Appendix B. Elflein et al. (2003) separated the esterified esters of chrysanthemum dicarboxylic acid (four isomers) by gas chromatography into two separated peaks (enantiomer peaks), probably the *cis* and *trans* isomers of the dicarboxylic acid (Table C17, Appendix C). Column and analysis conditions were not provided.

Permethrinic acid has two enantiomer pairs and four isomers ($2^n = 4$) (Table B33, Appendix B). The acid leaving group for permethrin, cypermethrin and cyfluthrin is permethrinic acid. The structure of this acid is given in Table 3. Angerer and Ritter (1997) separated the methyl esters of *cis*- and *trans*-permethrinic acid on a polysiloxane capillary column by GC (Table C18, Appendix C). The carboxylic acids of several of

these pyrethroids were also listed as *trans*- or *cis*-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylic acid. The acids may be separated on a CHIREX phase 3005 column (Phenomenex, 2320 W 205th Street, Torrance, CA 90501) by HPLC.

Esfenvaleric/fenvaleric acids are present in two isomeric forms as shown in Table B34, Appendix B. The structures of these acids are identical and displayed in Table 3. Li Z-Y et al. (2006) separated the methyl esters of the two isomers (R and S) on a chiral column by GC (Table C19, Appendix C).

Decamethrinic acid is the acid leaving group of deltamethrin, and exists in four isomeric forms as shown in Table B35, Appendix B. The acid possesses two bromines as shown in Table 3. The *cis* and *trans* methyl esters were separated from the esters of permethrinic acid by Angerer and Ritter (1997) by GC chromatography on a siloxane column (Table C20, Appendix C).

2.8 Alcohol Components

The alcohol moieties used in the synthesis of the 15 pyrethroids are shown in Table 4. A discussion of the synthesis of each alcohol (i.e., stereochemistry, etc.) is beyond the scope of this review, although allethrone and 5-(phenylmethyl)-3-furanmethanol have been mentioned in the discovery section. Information on the synthesis of α-hydroxy-α-(3-phenoxyphenyl) acetonitrile (R, S forms) (cyanohydrin of phenoxyphenyl aldehyde), and the resolution of the desired S form that produces (1R, 3R, α-S) deltamethrin may be

found in Chapter 2 of the Deltamethrin Monograph (1982). The alcohols and their metabolites are readily separated by partition chromatography on GC or HPLC columns. Ruzo and Casida (1977) and Ruzo et al. (1978, 1979) used TLC to separate and identify the alcohol metabolites.

[Insert Table 4 about here.]

2.9 Mammalian Toxicity

The mammalian toxicity of the pyrethroids is highly dependent upon the structure of the parent pyrethroid (e.g., does it have an α-cyano group, or not), nature of the isomer mixture, and whether one or more of the isomers has been enriched. Appendix B, Tables B1-B30, provide information on the nature of each of the pyrethroids listed in Table 5. For example, bioallethrin is composed largely of isomers A and B (46% each), whereas S-bioallethrin constitutes largely one isomer, B (> 90%). The administration of the pyrethroid in water vs. corn- or sesame-oil greatly decreases the rat oral toxicity (LD_{50}) of the pyrethroids.

[Insert Table 5 about here.]

Fig. 5, obtained from Wolansky and Harrill (2008), shows the acute toxicity (LD_{50}) values in rats for the parent pyrethroids, and one or more of their enriched isomers. Structural information on the compounds tested and their purity was limited to a few of

the more commonly used pyrethroids (i.e., allethrin, resmethrin, bifenthrin permethrin, deltamethrin, cypermethrin). The authors recognized the need to standardize test materials, the pyrethroid isomers used in the tests, and their purity for improving the value of information obtained from toxicological studies.

[Insert Figure 5 here.)

The Office of Pesticide Programs (OPP), EPA, Washington, DC, has a complete file on the acute and chronic toxicity of the registered pyrethroids. Information on these pyrethroids may be obtained from OPP through the "Freedom of Information Act." Some of the toxicology information on file in OPP is also available online in "Summary form" from the state of California's Department of Pesticide Regulation (CALEPA.ca.gov). The summaries are in *.pdf format, and may be downloaded. However, no information was available in this database for allethrin, cyhalothrin or tefluthrin, and the data involving tralomethrin were found under deltamethrin. The organization of the data base containing these files may be found in Knaak et al. (1993). The technical pyrethroid products (percent purity given) tested for toxicity were largely cis/trans mixtures (percentages given in the reports). No information was found concerning the nature of the individual isomers in the surveyed reports.

3 Experimentally-derived Absorption Parameters Used in PBPK/PD Models

The set of parameters shown in Table 1 are required to run the PBPK/PD models. In addition, the models for the organophosphate, carbamate and pyrethroid pesticide classes may require additional compartments and or parameters to properly predict the fate (absorption, distribution, metabolism, and elimination, or ADME) of pesticides in food, or amounts that will dissolve in a minimum volume of corn oil or polyethylene glycol 400 (PEG 400) for oral administration in toxicity tests. In general, pyrethroid insecticides that are ingested are rapidly and nearly completely absorbed by the small intestine, with small percentages being eliminated in feces and the remainder as metabolites in urine. In some cases, the events that occur are easily modeled and require a modest number of compartments (i.e., blood, brain, liver, kidney, rapidly and slowly perfused tissue, skin and GI tract) and parameters (i.e., partition coefficients, absorption, hepatic metabolism, metabolic and elimination rate constants). The pyrethroid insecticides (Type I/II) may require additional compartments (e.g., full gastrointestinal submodel) and parameters (e.g., protein plasma binding constants, plasma:tissue equilibrium dialysis constants, plasma partition coefficients, multidrug efflux P-glycoprotein (Pgp) permeability constants, and microsomal V_{max} and K_m values in liver or GI lumen) to properly model amounts ingested.

Environmental (e.g., surface and crop leaf residues) and work-place exposures (e.g., mixer-loaders, applicators, etc.) may result in the transfer of pyrethroids to skin where they may be absorbed. Dermal PBPK/PD models require compartment/submodels and additional parameters (e.g., K_p, permeation constants, exposed surface area, evaporation rates, washoff fractions, etc.) to model the fate of pyrethroids deposited on the skin.

The following sections address the development of parameters that are key to these models.

3.1 Gastrointestinal Absorption/Metabolism

A series of rate constants (k_s, h^{-1}) are currently used in physiologically-based models to describe the transfer of a dose to the stomach and then its distribution to the various regions of the small intestine. This compartmental approach was used by Timchalk et al. (2002) to predict the ADMET (absorption, distribution, metabolism, elimination and toxicity) of chlorpyrifos in rats and humans. Zhang et al. (2007) chose a compartmental transit and absorption GI model for carbofuran. The model incorporated the majority of the GI, including colon, duodenum, lower small intestine and stomach lumen (food flow, $L \cdot h^{-1}$, and volumes, L) and walls (volume, percent). Yu et al. (1996) developed one of the first compartmental absorption and transit (CAT) models that were described by a set of differential equations and kinetic rate constants. The division of the GI tract into seven regional pH-, volume- and permeability- compartments gave the best fit to the experimental results of the small intestinal's transit time vs. percent of dose that reached the colon.

The original CAT model (Yu et al. 1996) and modifications thereof (ACAT; Advanced Compartmental Absorption and Transit) by SimulationsPlus Inc., (Lancaster, CA) are being used to simulate the absorption of drugs through the gastrointestinal tract (Agoram et al. 2001). This dynamic physiological model, (ACAT), requires parameters (e.g.,

length, radius, transit time, and pH for GI tract, regional effective drug permeability (P_{eff}), solubility, absorption scale factors (ASF model; effect of log D values on absorption) and metabolism (enzyme, metabolic rate constants, and transporter expression)) for each compartment of the gastrointestinal tract (e.g., stomach, intestine and colon). Fig. 6 provides a schematic representation of an ACAT model for Talinolol (CAS no. 57460-41-0) (Tubic et al. 2006).

[Insert Figure 6 about here.]

The effective permeability (P_{eff}) in human intestine (GastroPlus Manual, version 6.0) may be calculated by measuring the rate of disappearance of a drug from a section of the GI tract at steady state as shown in Eq. [1]:

$$P_{eff} = \frac{Q(C_{in} - C_{out})}{2\pi RLC_{out}} \qquad [1]$$

where Q in the volumetric flow rate, C_{in} and C_{out} are the concentrations at the input and output posts, and R and L are the radius and length of the perfused section.

Eq. [1] may be rearranged to give:

$$\frac{dM}{dt} = P_{eff} * \alpha * M \qquad [2]$$

where

$$\alpha = \frac{(\text{cylinder surface}) * 2\pi RL}{(\text{cylinder volume}) * \pi R^2 L} = \frac{2}{R} \quad [3]$$

and

$$k_a = P_{eff} * \alpha \quad [4]$$

$$\frac{dM(i)_{absorbed}}{dt} = k_a'(i)V(i)[C(i)_{lumen} - C(i)_{enterocyte}] \quad [5]$$

where $dM(i)_{absorbed}/dt$ is the rate of absorption, $k_a'(i)$ is the absorption rate coefficient, $C(i)_{lumen}$ is the concentration in the lumen, $C(i)_{enterocyte}$ is the unbound concentration of drug in the enterocyte subcompartment, and i indicates a particular compartment.

Intestinal drug flux mediated by P-glycoprotein was measured in terms of flux by Stephens et al. (2001) as shown in the following equation (Eq. [6]):

$$J = \frac{J_{\min} + (J_{\max} - J_{\min})}{(1 + ((C / EC_{50})^{-P}))} \quad [6]$$

where J is the net flux, C is donor concentration, P is a constant (the Hill slope), and EC_{50} (K_m, μM) is the concentration at which half-maximal flux was achieved. J_{max} values range from 3.0 to 13.0 nmol $h^{-1}cm^{-2}$.

Permeability may be measured using Caco-2 cells or isolated intestine in Ussing chambers (Stephens et al. 2001). Caco-2 cell (immortalized human colon adenocarcinoma cell line) monolayers are widely used to evaluate the intestinal

absorption of drugs because they incorporate both passive transport (transcellular or paracellular route) and active transporters, such as the peptide transporter (PepT1), monocarboxylic transporter (MCT), and the efflux pump P-glycoprotein (P-gp) (Liang et al. 1995; Tamai et al. 1995; Ueda et al. 1986). The Caco-2 assay is a relatively low throughput method, as a result of its 3-week growth period and the requirement for regular maintenance feeding. The apparent permeability, P_{app}, values in $cm \cdot sec^{-1}$, was calculated by Irvine et al. (1999) using Eq. [7]:

$$P_{app} = \frac{dQ}{dt}\frac{1}{C_0}\frac{1}{A} \qquad [7]$$

where dQ/dt is the permeability rate (nmol s^{-1}), C_0 is the initial concentration (μM) in the donor compartment, and A is the surface area (cm^2) of the filter.

The use of a faster-growing cell line, MDCK (Madin-Darby canine kidney) cells, appears to be a good replacement for Caco-2 cells (Irvine et al. 1999). The parallel artificial membrane permeation assay (PAMPA) is a rapid in vitro assay, in which transcellular permeation is evaluated (Kansy et al. 1998). PAMPA may also be used to predict oral absorption, blood-brain barrier penetration, and human skin permeability (Fujikawa et al. 2007) by using QSAR models. To our knowledge, neither PAMPA, Caco-2 cell monolayers nor MDCK cells have been used to examine the absorption/permeability of the pyrethroids. The advantages and limitations of the Caco-2 model were reviewed by Artursson et al. (1996) and Delie and Rubas (1997).

3.2 Enterohepatic Circulation (EHC)

The need for dynamic enterohepatic circulation (EHC) models and parameters has been established for the carbamate pesticides (Zhang et al. 2007) and may also be needed for certain pyrethroids. Zhang et al. (2007) modeled the enterohepatic circulation of glucuronides formed during the metabolism of carbofuran in the rat. A bile to duodenum partition coefficient was used to describe the transfer of glucuronides from bile to the contents of the duodenum for reabsorption. Pyrethroid-based QSAR models are currently generating parameters for dynamic models involving liver metabolism (e.g., microsomal, CYP, and carboxylesterase (CaE) activity), percutaneous absorption, protein binding, and tissue-blood partitioning. These predictive QSAR models are addressed in the next sections along with models that involve toxicity.

3.3 Gut Metabolism/Transport

The total rate of gut metabolism includes all the enzymes (i.e., individual CYPs and CYP abundances, index i) that exist in the gut enterocyte compartments (index j) (Gastroplus 5.0, SimulationsPlus, Inc., Lancaster, CA). The CYP450 enzymes are present in the gut in smaller quantities (~20 times less) than in the liver; however, in some cases their contribution to metabolism is similar to what occurs in the liver. This is true for drugs that are highly bound to plasma proteins and have limited access to liver hepatocyte enzymes (Agoram et al. 2001). GastroPlus Manual version 6.0 describes the metabolic activity of all gut enzymes in Eq. [8].

$$\text{Gut Metabolic Rate} = \sum_{j}\sum_{i} \frac{V^{i}_{t\,\max} SF_{V_{\max}} ESF_{i,j} C^{j}_{ent} f_{ue}}{K^{i}_{m} SF_{K_m} + C^{j}_{ent} f_{ue}} \qquad [8]$$

where:

C_{ent} is the concentration of the drug in the enterocyte compartment *j*

f_{ue} is the unbound fraction of drug in the enterocytes

$V_{\max}$ is the maximum velocity, and K_m is the Michaelis-Menten constant (enzyme *i*)

$SF_{V_{\max}}$ and SF_{K_m} are the overall scale factors for all gut metabolic enzymes

ESF_{ij} are the gut enzyme distribution factors for enzyme *i* in compartment *j*

The same V_{max} and K_m from a hepatic enzyme may be used for the gut so long as the regional distribution of enzymes in the GI tract is expressed as an amount relative to the whole liver amount of the same enzyme.

Saturable carrier-mediated transport (P-glycoprotein):

Transport Rate = Influx – Efflux as described by GastroPlus Manual version 6.0, [Eqs. 9 & 10]

$$\text{Gut Influx Rate} = \sum_{j}\sum_{i} \frac{V^{i}_{\max} SF^{In}_{V_{\max}} ESF_{i,j} C^{j}_{lumen}}{K^{i}_{m} SF^{In}_{K_m} + C^{j}_{lumen}} \qquad [9]$$

$$\text{Gut Efflux Rate} = \sum_{j}\sum_{i} \frac{V^{i}_{\max} SF^{Ef}_{V_{\max}} ESF_{i,j} C^{j}_{ent} f_{e}}{K^{i}_{m} SF^{Ef}_{K_m} + C^{j}_{ent} f_{e}} \qquad [10]$$

where:

i = index for i-th influx/efflux transporter

j = index for j-th lumen compartment

$V_{max\ (i)}$ = maximum transport rate for influx/efflux transporter i

C^{j}_{lumen} = . concentration of drug in lumen compartment j

C^{j}_{ent} = concentration of drug in enterocyte compartment j

$K_{m(i)}$ = Michaelis-Menten constant (concentration at V_{max}) for transporter i

$ESF_{(i,j)}$ = scale factor for maximum transport rate for transporter i in compartment j

$SF^{Int}_{V\max}$ = overall influx transport scale factor

$SF^{Ef}_{V\max}$ = overall efflux transport scale factor

SF^{In}_{Km} = overall scale factor for K_m for influx transporters

SF^{Ef}_{Km} = overall scale factor for K_m efflux transporters

The efflux transport calculations are based on concentrations in the apical membrane of the enterocytes, while the influx transport calculations are based on concentrations in the lumen. Drugs that enter the enterocyte are subject to metabolism by CYP3A4, or those not so metabolized may be effluxed back into the lumen by P-glycoprotein to be reabsorbed and again metabolized.

In liver and kidney, P-glycoprotein is localized on the luminal membrane of hepatic canaliculi facing the bile duct lumen or on the luminal brush-border membrane of renal proximal tubular cells facing the renal tubule lumen. This means P-glycoprotein is

localized at the exit site of hepatocytes and renal epithelial cells and contacts only drug molecules after cellular uptake, intracellular distribution and metabolism in the liver and kidney. In contrast to the liver and kidney, P-glycoprotein is localized at the entrance site of epithelial cells of intestines. Drug molecules are exposed to P-glycoprotein prior to intracellular distribution and metabolism. The distribution of P-glycoprotein in the intestine strongly supports the role of P-glycoprotein in enhancement of CYP3A4 mediated metabolism.

P-glycoprotein appears to have a greater impact on limiting cellular uptake of drugs from blood circulation into brain and from the intestinal lumen into epithelial cells than on enhancing drug elimination from hepatocytes and renal tubules (Lin and Yamazaki 2003). The process of drug (e.g., vinblastine and digoxin) efflux via Caco-2 cells by P-glycoprotein has been demonstrated to be saturable, with Michaelis-Menten constants (K_m values) of 26 and 58 μM, respectively for vinblastine and digoxin (CAS no. 865-21-4 and 20830-75-5). Permeation studies indicate the following relationship:

Dose passing through intestinal cells = influx (passive diffusion and/or active uptake) – efflux and metabolism:

Drug elimination is enhanced by the formation of glucuronic acid conjugates. The uridine 5'-diphosphate-(UDP-)-glucuronosyltransferase (UGT) family of enzymes, comprising 14 functional "isoforms" (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2A1, 2B4, 2B7, 2B15, 2B17, and 2B28) catalyze the conjugation of chemicals containing a suitable nucleophilic atom (i.e., aliphatic or aromatic hydroxyl, carboxyl or amino groups) with

glucuronic acid. The structural features of substrates that confer isoform selectivity are not well understood. For almost all UGT isoforms, an aromatic ring attached to the nucleophilic atom increases the susceptibility of the nucleophilic atom to glucuronidation.

UGT is a membrane-bound protein with a poorly defined 3-dimension structure (Sorich et al. 2006). According to Chang and Benet (2005), the V_{max} and K_m constants for naphthol glucuronidation by human liver microsomes are 20.2 $nmol \cdot min^{-1} \cdot mg^{-1}$ and 216 μM, respectively. Little if any of the hydroxylated intact pyrethroids are conjugated prior to being eliminated. The importance of the activities of transporters and metabolizing enzymes in the ADME of pyrethroids has yet to be established. Inhibitors of these systems may be used to study their effect on ADME (Lau et al. 2003).

3.4 Skin Absorption

Until the beginning of the last century, the skin was believed to be an impervious layer, except perhaps for some permeability to inert gases (Scheuplein and Blank 1971). In 1877, Fleischer concluded that the intact skin of man was totally impermeable to all substances (Fleischer 1877). This belief was dispelled by Schwenkenbecker (1904) and others who demonstrated the relative permeability of skin to lipid-soluble substances and water.

3.5 Skin Permeability

It took almost 50 years after Schwenkenbecker's work to establish that the stratum corneum (horny layer) was the barrier that prevented water permeability of skin. The water permeability experiments of Berenson and Burch (1951) on isolated epidermis and stratum corneum provided evidence that the horny layer was, indeed, the permeability barrier. The permeability of water through skin is 0.2-0.4 $mg \cdot cm^{-2} \cdot h^{-1}$ at 30 ^{o}C according to Baker and Kligman (1967). The process of percutaneous absorption is described as being the adsorption onto the stratum corneum, diffusion through it and through the viable epidermis, and finally through the papillary dermis and into the microcirculation (Scheuplein and Blank 1971).The stratum corneum acts as a passive diffusion medium, with the degree of hydration, swelling, diameters of sweat ducts and hair follicles playing a role in the absorption process.

From using both in vivo or in vitro studies, human skin has been found to be more impermeable than the skin of other species such as the cat, dog, rat, mouse or guinea pig (Wester and Maibach 1977; Wester and Noonan 1980; Wester and Maibach 1993). Pig or guinea pig skin are favored for use in studies involving diffusion cells (Bronaugh et al. 1986; Harrison et al. 1984; Reifenrath and Kamppainen 1991) over other animal skin types, whereas skin from the forearm or back is favored in live monkey studies and in studies performed on human volunteers (Feldmann and Maibach 1974). The degree of percutaneous absorption has been determined by using the isolated perfused rabbit ear (Bast et al. 1997), and other models have employed porcine skin flaps for the same purpose (Riviere and Monteiro-Riviere 1991; Riviere et al. 1986; Nicoli and Santi 2007).

These methods are described in reviews authored by Bronaugh et al. (1982), Wester and Maibach (1993), and Barbero and Frasch (2009).

Work by Maibach et al. (1971), Serat (1973), Serat and Bailey (1974), and Serat et al. (1975) ignited interest in percutaneous studies involving worker exposure to cholinesterase-inhibiting organophosphorus pesticides in California. Two reviews by Knaak et al. (1989, 2002) detailed the history of the hazards posed by reentry into pesticide-treated foliage, laboratory and field exposure studies, and solutions for solving problems that included establishing pesticide reentry intervals in California. The use of blood cholinesterases as biomarkers of exposure in field studies was reviewed by Nigg and Knaak (2000). Ellison et al. (2011), Farahat et al. (2011), and Crane et al. (2011) reported on the effects of chlorpyrifos (CAS no. 2921-86-2) exposure to cotton-field workers in Egypt. Excellent agreement was obtained between cumulative urinary TCPy (CAS no. 6515-38-4) elimination, blood AChE and ButyrylChE inhibition, using a PBPK/PD model (Ellison 2011). The field workers in this Egyptian study were first exposed to chlorpyrifos, then cypermethrin (CAS no. 52315-07-8) and later profenofos (CAS no. 41198-08-7), during the 2008 pesticide application season. Workers having had contact with cypermethrin on their hands and arms during mixing and spraying reported a tingling sensation (Farahat, personal communication, 2009).

3.6 Relationship between Kp (permeation constant), Skin:Air, and Skin:Vehicle Partition Coefficients

The mathematical relationship between a dose applied to the skin and its absorption has been addressed by several authors (Scheuplein and Blank 1971; Guy et al. 1987; Potts and Guy 1992, 1995; Mattie et al. 1994; Moss et al. 2002; Geinoz et al. 2004) . The steady state flux (J_s, $\mu g \cdot cm^{-2} \cdot hr^{-1}$) of solute through a simple inert membrane is described in Eq. [11] in terms of Fick's equation.

$$Flux = Dk_m C / l = K_p C \qquad [11]$$

where D is the diffusion coefficient ($cm^{-2} \cdot h^{-1}$), K_m is the partition coefficient of the chemical in skin (unit less), C is the concentration of the chemical in skin ($\mu g \cdot cm^{-3}$), l is the thickness of the skin (cm), and K_p is the permeability constant ($cm \cdot h^{-1}$). Flux takes on units of $\mu g \cdot cm^{-2} \cdot h^{-1}$in the overall equation, depending upon whether or not C is in µg or µmoles, or some other unit of measurement.

For chemicals such as the glycol ethers (Boatman and Knaak 2001) or pesticides, and when vehicles such as water or air are used, skin:air and skin:water partition coefficients must be determined and used in this equation to account for the higher concentrations in skin caused by partitioning.

K_p, area, and concentration per unit area are routinely used in PBPK/PD models that describe the absorption of a dermal dose to workers (Ellison et al. 2011), as shown in Eq. [12]:

$$Dose(ug) = Area(cm^2) * C(ug * cm^{-3}) * K_p(cm * hr^{-1}) * time(hr) \quad [12]$$

3.7 Skin:Blood Partition Coefficients

Mechanistic models for determining the partitioning of a pesticide between skin and blood are addressed in Section 4.4, Tissue Partition Coefficients/Distribution. In this section, blood plasma protein binding (Fup) was included in the Poulin-Theil model (2000, 2002a,b) to calculate tissue:blood partition coefficients. This procedure greatly reduces the amount of unbound pesticide in blood that is available for partitioning into adipose tissues. A laboratory method, such as the one developed by Jepson et al. (1992, 1994) and reviewed by Knaak et al. (2008), may be used to determine whether predicted values are correct.

3.8 Dermal Dose-response, Pharmacokinetic, and Metabolism Studies

Sidon et al. (1988) developed 7 or 14 d dermal absorption data in Rhesus monkeys and Sprague-Dawley rats using permethrin (*cis* and *trans* isomers) labeled with ^{14}C in the alcohol and cyclopropyl groups. Permethrin was applied to the forehead or forearm of the monkey and to the mid-lumbosacral region of the rat. An intramuscular dose was employed to correct for urinary recovery. The forehead skin (24-28%) of the monkey was more permeable to both isomers than was forearm skin (14-21%). No significant difference in dermal absorption was observed between the *cis*- and *trans*- isomers. For

the rat, absorption was greater than in monkeys, with 46 and 43% of the *cis*- and *trans*-isomers being absorbed, respectively. In a similar study in six human volunteers, Wester et al. (1994) applied ^{14}C-pyrethrin in a formula containing 0.3% pyrethrin (5.5 $\mu g \cdot cm^{-2}$ of skin) and 3.0% of the insecticidal synergist, piperonyl butoxide, (75.8 $\mu g \cdot cm^{-2}$ of skin) to the ventral forearm. The forearms were washed with soap and water 30 min after the application. The absorbed dose was determined by analysis of urine samples collected over a 7-d period. The volunteers absorbed 1.9% of the applied dose of pyrethrin. The authors calculated that 7.5% of the pyrethrin dose applied to the scalp was absorbed.

The effect on skin absorption of several insecticides was tested after in vivo pretreatment of the skin with either a 3% fenvalerate or 3% parathion in ethanol solution (Chang et al. 1995). The insecticides tested were carbaryl, fenvalerate, lindane and parathion and their absorption was studied using a pig skin in vitro approach. Concentrations of 40 or 400 $\mu g \cdot cm^{-2}$ of carbaryl, fenvalerate, lindane and parathion were used. Pretreatment with an ethanol control, or fenvalerate and parathion increased the total absorption of these insecticides proportionally with dose. In a study by Hotchkiss et al. (1992), ethanol was again shown to increase the percutaneous absorption of chemicals.

In an in vitro percutaneous study was performed on rodent and pig skin with DEET (diethyl-m-toluamide; CAS no. 134-62-3), permethrin and carbaryl; results were that no permethrin was absorbed. It was believed by the authors that DEET inhibited the absorption of permethrin (Baynes et al. 1997). Bast et al. (1997) studied the percutaneous absorption of permethrin through the isolated perfused rabbit ear. Permethrin was applied

in isopropyl myristate (reference ointment) or in ethanol. The ethanol was evaporated off skin, and the skin was covered with 1.5 (w/w) methyl cellulose in water. The authors measured the appearance rates ($pmol \cdot min^{-1} \cdot cm^{-2}$) of 3-phenoxy benzene methanol (CAS no.13826-35-2) and 3-phenoxybenzoic acid (CAS no. 3739-38-6) in the effusate after dermal application (3.61 $\mu mol \cdot cm^{-2}$ of skin). No permethrin per se was found in the effusate. The metabolites were believed to be from impurities in the permethrin. From the concentration that permethrin constitutes in a pharmaceutical brand (Ambush®) and from detection limits, Bast et al. (1997) calculated a K_p of 2.63×10^{-8} $cm \cdot h^{-1}$.

Other investigators (Eadsforth et al. 1988; van der Rhee et al. 1989; Woollen et al. 1992) reported percutaneous absorption rates of 1% or more of applied cypermethrin, the metabolites of which appeared in urine, and may have originated from impurities in the cypermethrin.

Percutaneous absorption studies with cypermethrin by Scott and Ramsey (1987) indicated that 1% of an in vivo dose to rats was absorbed after an 8-h exposure; however, no absorption was detected in vitro using human and rat skin after 8 h. A K_p was not calculated from the information obtained in the in vivo study.

4 Mechanistic Models for Determining Tissue:Blood Partition Coefficients

4.1 Tissue Partition Coefficients/Distribution

Pyrethroid physiologically-based pharmacokinetic (PBPK) models require tissue:blood partition coefficients to describe the biological fate of chemicals in humans and animals. Partitioning into blood is determined by the solubility in the water and lipid components, and by protein binding. The effects of protein binding are more extensive in rat blood than in human blood for volatile organic chemicals of moderate or high lipophilicity (Payne and Kenny 2002). Compared with protein molecules in solution, as in blood, solid protein in tissues that have strong intermolecular interactions are expected to have a much reduced capacity for binding low molecular weight volatile organic compounds. The accuracy of such predictions is greater for human tissues than for rat tissues, because of the difficulties in modeling the very large protein binding effects in rat blood. In this regard, Payne and Kenny (2002) cautioned from their work that no single equation can be applied to all circumstances.

4.2 Partition Coefficients From In Vitro Data

Knaak et al. (2008) reviewed the literature pertaining to in vitro methods for developing tissue:blood partition coefficients for PBPK models. The experimental work of Jepson et al. (1992, 1994) is significant. Ultrafiltration was used to develop tissue:saline and tissue:blood partition coefficients. No new studies of this nature were found during the preparation of this review on pyrethroids. Automated laboratory methods are still needed and must be developed to insure the partition coefficients obtained from the use of mechanistic or QSAR models are reasonable (Payne and Kenny 2002; Rowland, personal communication 2010).

4.3 Selection of Po:w and Log Do:w Values

There are many log P/log D QSAR models (Knaak et al. 2008) published in the literature. The key to using the mechanistic (Poulin and Theil (2000, 2000a,b) approach to determining partition coefficients is the selection of a logP/logD model that adequately predicts values for the 15 pyrethroids targeted in this review, and their metabolites (ionized and nonionized species). Simulations Plus, Inc. (Lancaster, CA) uses built-in equations or data bases in GastroPlus to provide log P/log D values. On the basis of the findings in a carbamate review (Knaak et al. 2008), ACD Log D Suite, Version 12.0 (ACD, Advance Chemistry Development, Toronto, Ontario, Canada) was selected for predicting pKa, log P, log D, and water solubility (WS) values for the individual pyrethroids and their metabolites (see Appendix D and tables).

As part of the selection of a program for calculating log D, the WS for each of the pyrethroids and their metabolites were individually plotted against log $D_{pH7.4}$ values to determine whether a linear, but inverse, relationship existed, based on the knowledge that metabolism significantly increases the water solubility of administered pesticides, drugs and other chemicals. Water solubility was calculated using the equation [Eq. 13] of Meylan and Howard (1994a,b)

where:

$$\text{Log}(\text{S mol}\cdot\text{L}^{-1}) = 0.796 - 0.854\cdot\log(K_{o:w}) - 0.00728\cdot\text{MW} + \text{corrections} \quad [13]$$

ACD Log D values were used in the equation rather than log $K_{o:w}$ values to calculate water solubilities. This procedure yielded linear plots with r^2 values greater than 0.9 for all 14 pyrethroids and their metabolites. Figs. 7 and 8 show the plots for allethrin and cypermethrin. The numbers on the points in the two plots correspond to the numbers in Appendix D, Table D1, for allethrin and Table D5 for cypermethrin.

[Insert Figure 7 about here.]

Allethrin (pt 1) is at the bottom right hand corner of Fig. 7, while allethrone (pt 9) and allethrone glucuronic acid (pt 12), allethrone sulfate (pt 13) are at the top left hand corner with the glucuronic acids of Met B (pt 14) and Met A (pt 15). The unconjugated aglycones (pts 10, 11) appear below the conjugates. Point 4 belongs to allethrin acid.

[Insert Figure 8 about here.]

Water solubilities >10,000 ($g \cdot L^{-1}$) were calculated by the Meylan and Howard (1994a,b) equation for conjugated pyrethroids metabolites, but were not determined by the ACD Log D suite. Cypermethrin (pt 1) appears at the lower right hand corner of the plot followed by the ring hydroxylated cypermethrins (pts 2, 3, 4, 5 and 6). The metabolites in the middle of the plot are the cyanohydrins (pts 7, 8, 9 and 10), aldehydes (pts 11, 12, 13 and 14) and alcohols (pts 15, 16, 17 and 18). The metabolites at the upper end of the plot (pts 19-33) are the aminoacetic acids (glycine conjugates), benzoic acids, and conjugates of glucuronic and sulfuric acids.

4.4 Tissue:Blood Partition Coefficients

Knaak et al. (2008) used the Poulin and Theil (2000, 2002a,b) equations to obtain partition coefficients for a series of carbamate insecticides and their metabolites. Protein binding was not considered for carbamates because binding constants were not readily available. Binding was included for chemicals in the equations used by GastroPlus™, SimulationsPlus, Inc., Lancaster, CA (www.simulations-plus.com) (Berezhkovskly (2004a,b, 2007); Poulin and Theil (2000, 2002a,b); Rodgers et al.(2005, 2007), Rodgers and Rowland (2006, 2007)) to calculate partition coefficients, while SimCYP™ (www.simcyp.com) modified the Poulin and Theil (2000, 2002a,b) equations for nonadipose tissues from:

$$P_{t:p} = \frac{[P_{o:w}(V_{nlt} + 0.3V_{pht})] + [(V_{wt} + 0.7V_{pht})]}{[P_{o:w}(V_{nlp} + 0.3V_{php})] + [(V_{wp} + 0.7V_{php})]} x \frac{f_{up}}{f_{ut}} \quad [14]$$

to:

$$P_{t:p} = \frac{[P_{o:w}(V_{nlt} + 0.3V_{pht})] + [(V_{wt} / f_{ut} + 0.7V_{pht})]}{[P_{o:w}(V_{nlp} + 0.3V_{php})] + [(V_{wp} / f_{up} + 0.7V_{php})]} \quad [15]$$

and for adipose tissue from:

$$P_{t:p} = \frac{[D_{vo:w}(V_{nlt} + 0.3V_{pht})] + [(V_{wt} + 0.7V_{pht})]}{[D_{vo:w}(V_{nlp} + 0.3V_{php})] + [(V_{wp} + 0.7V_{php})]} x \frac{f_u}{1} \quad [16]$$

to:

$$P_{t:p} = \frac{[D_{vo:w}(V_{nlt} + 0.3V_{pht})] + [V_{wt} + 0.7V_{pht})]}{[D_{vo:w}(V_{nlp} + 0.3V_{php})] + [(V_{wt} / f_{up} + 0.7V_{php})]} \quad [17]$$

based on the work of Berezhkovskly (2004a,b, 2007).

The Berezhkovskly (2004b) equation for obtaining tissue:plasma and tissue:blood partition coefficients is as follows:

$$P^0_{t-p} = [K_{vo-w}(V_{tn} + 0.3V_{tph}) + 0.7V_{tph} + V_{tw} / F_{ut}] / [K_{vo-w}(V_{pn} + 0.3V_{pph}) + 0.7V_{pph} + V_{pw} / F_{up}] \quad [18]$$

where F_{ut} and F_{up} are the unbound (free) drug fractions for tissue and plasma. The derivation of equation (1.5) is given in the paper by Berezhkovskly (2004b). Berezhkovskly points out that his definition of F_{ut} and F_{up} differs from the commonly used definitions f_{ut} and f_{up} of unbound fractions in tissue and plasma. The parameter F_{ut} describes only the protein binding through the values of the equilibrium dissociation constants K_{dj} (k_{dj}^{-} / k_{onj}) and protein concentrations P_j, while f_{ut} and $f_{up,}$ include both protein binding and partitioning. Equations 18-20 describe the relationships for F_{ut} and $f_{ut.}$

$$F_{ut} = 1/(1+\sum P_j/K_{dj}) \quad [19]$$

where,

$\sum P_j / K_{dj} = \sum k_{tj}^{+} / k_{tj}^{-} = 1/F_{ut} - 1$, and $k_{tj}^{+} = k_{onj} P_j$

while the equation for f_{ut} is as follows:

$$f_{ut} = 1/(\sum P_{tj-w} v_{tj} + v_{tw}/F_{ut}) \text{ [20]}$$

where

$v_{tw} = V_{tw}/V_t; v_{tj} = V_{tj}/V_t$ and $P_{tj-w} = (k_{tj}^{+}/k_{tj}^{-})(v_{tw}/v_{tj})$.

In summary, the tissue-plasma partition coefficient calculated in Eq. [21] takes us back to Eq. [18] in the Berezhkovskly paper.

$$P_{t-p}^{o} = f_{up}/f_{ut} = (\sum P_{tj-w} v_{tj} + v_{tw}/F_{ut})/(\sum P_{pj-w} v_{pj} + v_{pw}/F_{up}) \text{ [21]}$$

On the basis of the work by Berezhkovskly, Poulin-Theil and the GastroPlus User Manual (SimulationsPlus, Inc., Lancaster, CA), the binding equations (Eqs. 22 and 23) for the input parameters (f_{up}, f_{ut}, f_{up}/f_{ut}) were added to Table 6 for calculating partition coefficients for the 15 pyrethroids and their metabolites that we have targeted in this review. Table 6 was derived from Table 6 in Knaak et al. (2008). Eq. [22] for calculating f_{up} was obtained from the GastroPlus User Manual. This equation requires values for F_{up} (% unbound to plasma protein). F_{up} values were determined using a QSAR program for predicting K_{hsa} values for the pyrethroids and their metabolites (Chang 2009).

$$f_{up} = \frac{1}{10^{\log D_{7.4}}(\frac{V_{lipid}}{V_{water}}) + 1 + \frac{1 - F_{up,\exp}}{F_{up,\exp}}} \text{ [22]}$$

$$f_{ut} = \frac{1}{(1 + \{[(1 - f_{up})/f_{up}] x R A_t\})} \text{ [23]}$$

RA_t = the ratio of albumin concentration found in tissue over plasma. When using Poulin's method for adipose tissue, we assume that $RA_t = 0.15$; for other tissues we assume that $RA_t = 0.5$.

Eq [23] for calculating f_{ut} was already available in the Excel spreadsheet (Table 6; Knaak et al. 2008) used to calculate the Poulin-Theil partition coefficients for the carbamates Knaak et al. (2008). To correct for protein binding, the nonadipose and adipose tissue equations (14 and 16) of Poulin-Theil were both multiplied by f_{up}/f_{ut}. The revised Excel spreadsheets were used to calculate rat and human partition coefficients for the pyrethroids and their metabolites in Appendix D.

The users of the GastroPlus 5.0 program (SimulationsPlus, Inc., Lancaster, CA) are provided with a series of methods for calculating partition coefficients for perfusion- and permeability-limited tissues, when running PBPK models. The Poulin and Theil (homogeneous and extracellular), Berezhkovskly and Rodgers, Leahy, and Rowland methods for calculating partition coefficients are among those available in the program. The Poulin and Theil and Berezhkovskly models take into consideration the volume fractions of water, phospholipids and neutral lipids, unbound fractions in lipid non-depleted tissues and plasma (fu_t and fu_p), and unbound fractions in tissue and plasma proteins (Fu_t and Fu_p). The Poulin and Theil models per se do not take into consideration the unbound fractions in tissue and plasma protein (Fu_t and Fu_p).

[Insert Table 6 about here.]

Rodgers et al. (2005, 2007) and Rodgers and Rowland (2006, 2007) incorporated drug ionization and the interaction of strong bases with acidic phospholipids in calculating partition coefficients (K_p) for drugs, where $K_p = K_{pu} * f_u$. The basic equation for the calculation of unbound tissue-plasma partition coefficient, K_{pu}, is shown in Eq. [24] as it has been rewritten by Simulations-Plus, Inc., Lancaster, CA in their GastroPlus user manual (version 6.0).

Strong bases and group I zwitterions (compounds with at least one base pKa ≥ 7)

$$K_{pu} = V_{ewt} + \left(\frac{(1/X_{[D],IW})}{(1/X_{[D],P})} V_{iwt}\right) + \left(\frac{K_a \cdot [AP]_T ((1/X_{[D],IW}) - 1)}{(1/X_{[D],P})}\right) + \left(\frac{K \cdot V_{nlt} + (0.3K + 0.7) V_{pht}}{(1/X_{[D],P})}\right) \quad [24]$$

where:

V_{nlt}, V_{pht}, V_{ewt}, V_{iwt} = volume fraction of neutral lipids, phospholipids, intracellular and extracellular water in tissues.

K_a = association constant of basic compound with acidic phospholipids.

$[AP]_T$ = concentration of acidic phospholipids in individual tissues.

$X_{[D],IW}$, $X_{[D].P}$ = fraction of neutral drug species in intracellular space (pH = 7.0) and plasma (pH = 7.4)

K = solvent/water partition coefficient for the drug (vegetable oil/water partition coefficient for adipose and yellow marrow; 1-octanol/water partition coefficient for all other tissues).

The term $\left(\frac{Ka\left[AP\right]_T\left(\left(1/X_{[D],Iw}\right)-1\right)}{\left(1/X_{[D],P}\right)}\right)$ represents the interactions of bases with acidic phospholipids.

The equation for the calculation of unbound tissue-plasma partition coefficients, $K_{pus,}$ for neutral pyrethroids and their acidic metabolites is shown in Eq. [25].

Acids, neutrals, weak bases and group 2 zwitterions (all pK_{as} <7)

$$K_{pu} = \frac{(1/X_{[D],IW})V_{iwt}}{(1/X_{[D],p}} + V_{ewt} + (\frac{K \cdot V_{nlt} + (0.3K + 0.7)V_{pht}}{(1/X_{[D],p})} + [(\frac{1}{f_{up}} - 1 - \frac{K \cdot V_{nlp} + (0.3K + 0.7)V_{php}}{(1/X_{[D],p})})xRA_t \quad [25]$$

where:

V_{nlt}, V_{pht}, V_{ewt}, V_{iwt} = volume fraction of neutral lipids, phospholipids, intracellular and extracellular water in tissues.

V_{nlp}, V_{php} = volume fraction of neutral lipids and phospholipids in plasma.

RA_t = the ratio of albumin (for non-ionizable compounds) or lipoprotein (for ionizable compounds) concentration found in tissue over plasma.

$X_{[D],IW}$, $K_{[D],p}$ = fraction of neutral drug species in intracellular space (pH = 7) and plasma (pH = 7.4).

K = solvent/water partition coefficient for the drug (vegetable oil/water partition coefficient for adipose and yellow marrow; 1-octanol/water partition coefficient for all other tissues).

f_{up} = fraction of unbound in plasma

For all types of compounds, the final K_p is calculated as:

$$K_p = K_{pu} * f_{up}$$

Two equations [24, 25] were combined in GastroPlus by SimulationsPlus, Inc. into a single equation [26] to provide a continuous shift from neutral and acid compounds, which bind almost exclusively to albumin, to strong bases that bind almost exclusively to acidic phospholipids,

$$K_{pu} = V_{ew} = \frac{1/X_{[D],iw}}{1/X_{[D],p}} V_{iw} + \left(\frac{P \cdot V_{nlt} + ((0.3 \cdot P + 0.7) \cdot V_{pht})}{1/X_{[D],p}}\right) +$$
$$(F_n + F_a) \cdot \left[\left(\frac{1}{f_{up}} - 1 - \left(\frac{P \cdot V_{nlp} + (0.3 \cdot P + 0.7) \cdot V_{php}}{}\right)\right] \cdot RAtp + \quad [26]$$
$$(F_c) \cdot \left(\frac{K_a \cdot [AP]_T ((1/X_{[D],IW}) - 1}{(1/X_{[D],p}}\right)$$

where ($F_n + F_a$) is the fraction of drug without positive charge in plasma and F_c is the fraction of drug with positive charge in plasma. The remaining parameters have the same meaning as in the original two Rodgers equations.

4.5 Partition Coefficients for Pyrethroids and Metabolites

The spreadsheet in Table 6 was used to calculate the partition coefficients for allethrin and cypermethrin which are depicted in Figs. 9 and 10 (Appendix D, Tables D1 and D5).

Graphs of log $D_{pH7.4}$ vs. Adipose Tissue:Blood and Liver:Blood Partition Coefficients:

The plots of log $D_{o:w\ (pH\ 7.4)}$ vs. $PC_{t;b\text{-}liver}$ and log $D^*_{vo:w\ (pH\ 7.4)}$ vs $PC_{t:b\text{-}adipose}$ for allethrin and cypermethrin are shown in Figs. 9 and 10. In these two Figs., the water-soluble metabolites, with log $D_{pH7.4} < 2$, all have $PC_{t:b\text{-}liver}$ and $PC_{t:b\text{-}adipose}$ values ≤ 2.0 at the bottom of each plot; in contrast, the parent pyrethroid and their neutral metabolites, with log $D_{pH7.4} > 0$, have $PC_{t:b}$ values ranging from 1.0 to 80.0 extending up in a sigmoid curve to the right side of each plot.

[Insert Figures 7 and 8 about here.]

[Insert Figures 9 and 10 about here.]

5 In Vivo Metabolism of 15 Pyrethroid Insecticides

The metabolic pathways for each of the 15 pyrethroids addressed in this review are given in Tables E1-E17 (see Appendix E). The tables were developed from available pathway data published in the literature (Anadon et al. 1996; Cole et al. 1982; Crawford and Hutson 1977; Hutson and Logan 1986; Izumi et al. 1984; Kaneko et al. 1987; Kaneko and Miyamoto 2001; Miyamoto et al. 1974; Nakamura et al. 2007; Ruzo et al. 1978; Staiger and Quistad 1984; Tomigahara et al. 1994a,b,c, 1996, 1997; Ueda et al. 1975) and from physiological models (Mirfazaelian et al. 2006) developed from the pathways.

A preliminary physiological model for allethrin is presented in Fig. 1F, Appendix F. Preliminary ACSL-based physiological models were also written for deltamethrin, *cis*/*trans* permethrin, and *cis*/*trans* resmethrin (Knaak, unpublished, 2010). A PBPK model was developed for deltamethrin (Mirfazaelian et al. 2006; Godin et al. 2010; Tornero-Velez et al. 2010), to assess internal dose levels in various organs and tissues of rats and humans. Deltamethrin was selected to represent the disposition of the pyrethroids, because it is structurally similar to other commercial pyrethroids and is formulated as a single isomer ((S)-cyano-3-phenoxybenzyl (1R, 3R)-cis-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carboxylate). This minimized any complexities introduced by the presence of multiple isomers. An important characteristic of these models is that they incorporate diffusion-limited kinetics. Godin et al. (2010) proposed

diffusion-limited kinetics in all tissues, while Mirfazaelian et al. (2006) and Tornero-Velez et al. (2010) proposed mixed flow- and diffusion-limited kinetics. Based on a finding of apparent first-order kinetic behavior, Godin et al. (2010) applied a first-order clearance structure in modeling the biotransformation of deltamethrin.

In the past, industry sold most pyrethroids as isomer mixtures; however, in recent years the active isomer or isomers are being made and sold under different, but similar product names (e.g., biopermethirn, cismethrin, bioresmethrin and deltamethrin). This reflects the fact that the manufactures were able to economically produce one, or primarily one isomer. It is, therefore, reasonable to now develop PBPK models for these active isomers, rather than to try to construct them to only reflect the metabolism of mixtures. As a result of our having reviewed the metabolism of the 15 pyrethroids of interest in this paper, we have attempted to point out the isomers of each product that were studied (e.g., labeled with ^{14}C), and to provide their relative insecticidal activities. In Appendix D, Tables D1-D15, we present the chemical structures (generic), physical parameters, and tissue partition coefficients of the parent pyrethroids and their metabolites. The stereochemistry of these pyrethroids was presented in Appendix B (see Tables B1-B30).

5.1 Allethrin

Casida et al. (1971a,b); Yamamoto et al. (1971); and Elliott et al. (1972) analyzed the rat urinary metabolites resulting from the administration of ^{14}C-allethrin (CAS no. 584-79-2). Allethrin was oxidized at the chrysanthemate isobutenyl moiety to a *trans*-2-carboxy-

1-propen-1-yl group and at the allyl group to derivatives of 1-hydroxy-2-propenyl-1-yl and 2, 3-dihydroxypropyl. A methyl group on the cyclopropyl moiety was hydroxylated to give the hydroxymethyl derivative. Chrysanthemum dicarboxylic acid (CAS no. 497-95-0) and allethrolone (CAS no. 29605-88-7) were found as hydrolysis products in urine. The major metabolites in rats were the diol of allethrin carboxylic acid (CAS no. 31338-05-3), allethrin monohydroxy acid (CAS no. 31338-06-4) and allethrin hydroxymethyl acid (CAS no. 36912-77-3). The last two metabolites were found by Class et al. (1990) in urine to partly exist as conjugates. Metabolites were tentatively identified by chemical ionization mass spectrometry, following treatment with diazomethane or diazoethane and trimethylsilylacetamide and high-resolution GC. According to Yamamoto et al. (1971), allethrin is eliminated primarily as intact esters, with small amounts of the chrysanthemum dicarboxylic acid being present in urine. In Table D1, Appendix D, we give the structure and technical name of allethrin. No information was found concerning the stereochemistry of the allethrin used in the metabolic studies and the position of the label(s).

5.2 Bifenthrin

A complete metabolic pathway for bifenthrin in the rat is given in Table D2, Appendix D. Intact bifenthrin is first hydroxylated on the biphenyl ring of one of the dimethyls on the cyclopropane ring before being hydrolyzed to yield alcohols (e.g., CAS no. 76350-90-8) and TFP acid (CAS no.74609-46-4). This metabolism information was obtained from Fig. 58.2 and 76.2, Kaneko and Miyamoto (2001) and Kaneko (2010), respectively.

Bifenthrin and cyhalothrin both posses the 2-chloro-3, 3, 3-trifluoropropenyl group; however, they differ by cyhalothrin having a cyano group and a phenoxyphenyl ring, while bifenthrin has a 1, 1'-biphenyl ring and no cyano group. Bifenthrin (100% *cis*), contains two isomers (A + D), and is the primary commercial product in use. No information was given by Kaneko (2010) regarding the stereochemistry of the bifenthrin used in the metabolism studies.

In a rat study involving the two isomers of bifenthrin, the major metabolites found in rat plasma (Smith et al. 2002; Tullman 1987) were the parent compound, the hydrolysis product, 2-MBP alcohol, [1'1-biphenyl]-3-methanol, 2-methyl (CAS no. 76350-90-8), and the oxidized product of the alcohol, 2-MBP acid, [1,1'-biphenyl]-3-carboxylic acid (CAS no. 115363-11-6). The 2-MBP acid compound is analogous to 3-phenoxybenzoic acid (CAS no. 3739-38-6), the hydrolysis product of cypermethrin, deltamethrin, permethrin and fenvalerate (Huckle et al. 1981a,b, 1984; IARC 1991; Woollen et al. 1992). In addition to these ester-cleaved products, 4'-OH, 2-MBP alcohol (CAS no. 115340-46-0) and 4'-OH, 2-MBP acid (CAS no. NA) were found. According to Kaneko (2010), these metabolites are metabolically converted to dimethoxy 2-MBP alcohol and dimethoxy 2-MBP acid.

In another study (Wheelock et al. 2006), the 2-methyl group of bifenthrin was reported to be hydroxylated to give 2-hydroxymethyl bifenthrin. The 4'- and 2'-positions of 1, 1' biphenyl were also hydroxylated to give the 4'OH and 2'OH pyrethroids before

hydrolysis by carboxylesterases. According to Wheelock et al. (2006), bifenthrin is resistant to attack by carboxylesterases.

5.3 Cyfluthrin

The metabolic pathway data presented in Table D3, Appendix D, and Table E3, Appendix E, were obtained from Figs. 58.4 and 76.4 in Kaneko and Miyamoto (2001) and Kaneko (2010), respectively. The structure and stereochemistry of cyfluthrin is given in Table 2. Cyfluthrin is a Type II pyrethroid, and possesses a cyano group. β-Cyfluthrin (CAS no. 68359-37-5) contains both the *cis* (30-40%) and *trans* (57-67%) isomers of cyfluthrin and is the primary commercial-use product. The *cis* and *trans* forms each contain two isomers (B+G) and (D+E) as shown in Appendix B, Table B5. The other four forms (A+H) and (C+F) comprise ~5% of β-cyfluthrin. No information was given by Kaneko (2010) regarding the stereochemistry of the cyfluthrin used in metabolic studies.

In rat studies, the 4-fluoro-3-phenoxylphenyl group of cyfluthrin is hydroxylated in the 2'-, 4'- and 5'-position, while one of the 2, 2-dimethyl groups is hydroxylated to form a 2-hydroxymethyl derivative. Hydrolysis of the ester(s) yields the cyanohydrins of phenoxybenzeneacetonitrile (e.g., CAS no. 76783-44-3), which are further converted to alcohols, acids and ultimately are conjugated with glucuronic, sulfuric acid or glycine. The liberated dichlorocyclopropane-carboxylic acids (DCCA; 3-(2, 2-dichloroethenyl)-2, 2-dimethyl-cyclopropanecarboxylic acid, and 2-OHMeDCCA; 3-(2, 2-dichloroethenyl)-

2-(hydroxymethyl)-2-methyl-cyclopropanecarboxylic acid) are eliminated *per se,* or as conjugates with glucuronic acid prior to excretion in urine.

In a human inhalation study by Leng et al. (1997), nine male volunteers were exposed to an aerosol containing cyfluthrin at mean concentrations of 160 and 40 $\mu g/m^3$. The main urinary metabolites of cyfluthrin were *cis*/*trans* DCCA and 4-fluoro-3-phenoxybenzoic acid (FPBA). The same metabolites were found in the urine of pesticide applicators applying cyfluthrin in Germany (Hardt and Angerer 2003).

The urinary biomarker FPBA was also found in the urine of humans exposed to indoor residential cyfluthrin residues during a Jazzercise™ exercise program (Williams et al. 2003).

5.4 Cyhalothrin

The nature of the metabolites of cyhalothrin are listed in Table D4, Appendix D, and are shown in Figs. 58.5 and 76.5 as taken from Kaneko and Miyamoto (2001) and Kaneko (2010), respectively. γ-Cyhalothrin (CAS no. 76703-62-3) is the most active insecticidally of the 8 possible chiral isomers (16 involving the double bond) of cyhalothrin. Cyclopropyl-C^{14} labeled γ-cyhalothrin (position 1) was prepared by Johnson (2007) for studying the metabolism and the environmental fate of this pyrethroid. This pyrethroid is currently marketed by Pytech Chemicals GmbH (joint venture between Dow AgroSciences and Cheminova A/S). Information on the metabolism of cyhalothrin and bifenthrin is scanty. According to the literature, the CF_3 (dihalovinyl) group is stable and the double bond (vinyl group) does not undergo hydroxylation. Cytochrome P450

enzymes hydroxylate the alcohol (benzenemethanol, 2-phenoxy-) in the 4- position and perhaps in the 2-position, and in the 5'-position as well, before hydrolysis by carboxylesterases.

According to Kaneko (2010), the major metabolic reaction is ester hydrolysis followed by hydroxylation of the alcohol moiety and its subsequent oxidation to an acid. The cyano group of the alcohol, benzeneacetonitrile, α-hydroxy-3-phenoxy (CAS no. 39515-47-4) is converted to an SCN ion. The major metabolites of the acid moiety are cyclopropylcarboxylic acid and its glucuronide, while the alcohol moiety is converted to PB acid (benzoic acid, 3-phenoxy) (CAS no. 3739-38-6), 4'-OH PB acid (benzoic acid, 3-(4-hydroxyphenoxy) (CAS no. 35065-12-4), and the sulfate of 4'-OH-PB acid (benzoic acid, 3-[4-(sulfooxy)phenoxy]) (CAS no. 58218-91-0).

5.5 Cypermethrin

Table D5, Appendix D, shows the metabolites of cypermethrin as defined by Kaneko and Miyamoto (2001) and Kaneko (2010). Cypermethrin (8 stereoisomers) typically contains 20-40% α-cypermethrin (CAS no. 67375-30-8; B+G isomers). The two isomers, B+G are the most toxicologically active isomers in cypermethrin.

Crawford et al. (1981a) orally administered separate *cis* and *trans* isomer mixtures or *cis-trans* mixtures of radiolabeled cypermethrin (cyclopropyl-^{14}C, benzyl-^{14}C or cyano-^{14}C) individually to male and female rats (1-5 mg·kg^{-1}), in a mass balance and tissue retention

study. The cypermethrin isomers were analyzed on a column of 5-µm Hypersil (Shandon Southern Products Ltd., Runcorn, U.K.) (20 cm x 4.5 mm) using hexane in 30% water-saturated dichloromethane (4:1 v/v).The isomers eluted as follows: 1R, *cis*-αR and 1S, *cis*-αS isomers; 1R, *cis*-αS and 1S, *cis*-αR isomers; 1S, *trans*-αS and 1R, *trans*-αR isomers; 1S, *trans*-αR and 1R, *trans*-αS isomers. Single oral doses of *cis*- and *trans*-cypermethrin (benzyl-^{14}C label) were rapidly eliminated in urine with the exception of the *cis* isomer in female rats, in which the 0-24 output was 53% for males and 35% for females. A sex difference was not observed for the *trans*-isomers.

A total of 98.9% of the label from the *cis*-isomers was recovered in 8 d and 103.2% for the *trans*- isomers was recovered in 3 d. No respiratory $^{14}CO_2$ was eliminated. The radioactivity from (cyclopropyl-^{14}C)-*cis*, *trans*-cypermethrin (mixture) was eliminated in the urine of both male (55.8%) and female (66.5%) rats. Fecal elimination amounted to 28.7% by male and 27.0% by female rats. Less than 0.1% of the dose was eliminated as respiratory carbon dioxide. The radioactivity from the (cyano-^{14}C)-*cis*, *trans*-cypermethrin (mixture) was different from the other two labels as the α-cyano group was released and converted to thiocyanate ion, which was slowly eliminated from the body. The majority of the administered radiocarbon was recovered in the feces (42.0-57.2%) with lesser amounts in urine (8.3-9.6%). Respiratory carbon dioxide contained 1-2% of the administered radioactivity. Tissue residues from (benzyl-C^{14}) *cis*-cypermethrin were highest in fat (0.83-1.46 µg/g) of male and female rats, 8 d after administration.

Information on the nature of the excreted metabolites was published by Crawford et al. (1981b). The major metabolic reactions were hydrolysis of *trans*- and *cis*-cypermethrin, hydroxylation of a geminal dimethyl on the cyclopropane ring, and hydroxylation of the phenoxy group at the 4'-position. The unlabeled standard compounds used for metabolite identification were described by Ruzo and Casida (1981), Ruzo et al. (1978), and Unai and Casida (1977). Separation and identification of metabolites was achieved on F254 silica gel 60 TLC chromatoplates (Cole et al. 1982).

In the just mentioned 1982 study by Cole et al., metabolites from ^{14}C-acid (geminal dimethyl), ^{14}C-alcohol (benzyl methine) and ^{14}CN (cyano) labeled (1R, αS)-cis cypermethrin preparations and their percentages were identified in urine and feces. Cole et al. (1982) recovered 100% of a ^{14}C-benzyl labeled dose (0.12 mg/kg) of cypermethrin [(1R, αS)-cis cypermethrin, [CAS no. 65731-84-2] in the urine (62.9%), feces (33.8%) and tissue (3.3%) of rats. Cypermethrin is metabolized in the rat to the 4'-OH ester (CAS no. 64691-63-0) (4.1%), PB acid (CAS no. 3739-38-6) (5.9%), 4'-OH PB acid (CAS no. 35065-12-4) (3.0%), the glucuronides of PB alcohol (CAS no. 65658-93-7) (15.4%), PB acid (CAS no. 57991-35-2) (0.1%), 4'-OH PB acid (CAS no. 66858-01-7) (5.4%), the sulfates of 4'-OH PB acid (CAS no. 58218-91-0) (28.7%), 2'-OH PB acid (CAS no. 61183-26-4) (3.3%), and the glycine conjugate of PB acid (CAS no. 57991-36-3) (1.5%). Products retaining the ester linkage were found in feces, while hydrolysis products were present in urine. The findings by Cole et al. (1982) are in agreement with those of Crawford et al. (1981a,b) and Ruzo et al. (1978).

Hutson and Logan (1986) reported on the metabolic fate in rats of WL85871, which was a mixture of two isomers of cypermethrin [(1R, *cis*) αS and (1S, *cis*) αR]. Approximately 20% of the ingested compound was not absorbed and was eliminated unchanged in the feces. There was no evidence for any racemization of the chiral centers of WL85871 either in the intestine, the feces, or in fat. Approximately 40% of a 2.0 mg/kg dose was eliminated in urine as 3-(4-hydroxyphenoxy) benzoic acid sulfate (CAS no. 58218-91-0). Some hydroxylation occurred before hydrolysis. The small proportion of the dose stored in adipose tissue was eliminated by biphasic kinetics (2-3 d and 17-26 d).

Humans eliminated conjugates of 3-phenoxybenzoic acid (CAS no. 3739-38-6), 3-(4'-hydroxyphenoxy) benzoic acid (CAS no. 35065-12-4) and cyclopropanecarboxylic acid (DCCA; CAS no.55701-05-8) in urine according to Woollen et al. (1992). In the Woollen et al study, cypermethrin was administered orally to six male volunteers as a single dose (3.3 mg; cis:trans 1:1) and dermally to six volunteers at a dose of 31 mg/800 cm^2 (cis:trans 56:44) of skin. Cypermethrin was orally absorbed between 27-57% (mean of 36%) based on the elimination of DCCA and four times greater based on the recovery of benzoic acid conjugates. In the case of the dermal studies, 1.2% of the applied dose was recovered in urine as benzoic acid conjugates.

5.6 Deltamethrin

Table D6, Appendix D, gives the metabolites of deltamethrin as presented in Fig. 58.8 and 76.8, respectively, by Kaneko and Miyamoto (2001) and Kaneko (2010).

Deltamethrin (CAS no. 52918-63-5) is sold as a single chiral isomer [(1R, αS)-cis deltamethrin].

Cole et al. (1982) recovered 100% of a ^{14}C-benzyl labeled dose of deltamethrin [(1R, αS)-*cis* deltamethrin, CAS no. 52918-63-5] administered to rats (0.32 mg/kg), in urine (65.2%), feces (32.6%) and tissues (2.2%). These recovery values are quite similar to those obtained using radiolabeled cypermethrin. Metabolites from acid-^{14}C, alcohol-^{14}C and ^{14}CN-labeled preparations and their percentages were identified in urine and feces by Cole et al. (1982). Products retaining ester linkage were found in feces, and hydrolysis products were present in urine. The Cole et al. (1982) findings are in agreement with those of Crawford et al. (1981a,b) and Ruzo et al. (1978). Staiger and Quistad (1984) reviewed the nature of the urinary metabolites of deltamethrin resulting from the administration of 0.32 mg·kg^{-1} to rats. The same alcohol-^{14}C- and ^{14}CN-labeled metabolites were eliminated by rats receiving deltamethrin (0.32 mg·kg^{-1}) as those receiving cypermethrin (0.12 mg·kg^{-1}). The percentages of each of the metabolites varied somewhat, particularly by showing differences in the percentage of each of the glucuronides or sulfates. The glucuronide of PB alcohol from deltamethrin was lower (4.1% vs. 15.4%) than the corresponding one from cypermethrin. The sulfate of 4'-OH PB acid arising from the administration of deltamethrin was higher (47.5%) than from cypermethrin (28.7%). The significance of this difference is unknown and may not be very important, because overall recoveries and urinary elimination were very similar.

5.7 Fenvalerate

Table D7, Appendix D, gives the chemical structure of parent pyrethroid and metabolites of fenvalerate (Kaneko and Miyamoto 2001; Kaneko 2010). Fenvalerate or Pydrin (CAS no. 51630-58-1) contain equal amounts of the 4 chiral isomers (Appendix B, Table B14), while esfenvalerate (CAS no. 66230-04-4), shown in Table B14, is comprised of 84% of the D isomer.

Ohkawa et al. (1979) recovered 100% of α-^{14}C-benzyl labeled dose of fenvalerate (CAS no. 51630-58-1), administered to rats (8.0 mg/kg), in urine (63.9%), feces (31.7%) and tissue (4.4%) at the end of the study. Staiger and Quistad (1984) identified the urinary metabolites of fenvalerate resulting from the administration of 8.0 mg·kg^{-1} to rats. The primary metabolites were the 4'-OH ester (5.6%), the sulfates of 4'-OH PB acid (40%) and 2'-OH PB acid (3.7%) and unconjugated aglycones (5.2%). The glucuronides of these acids and PB acid were present at considerably lower percentages (~2.3%).

5.8 Fenpropathrin

Table D8, Appendix D, provides the chemical structures of parent pyrethroid and metabolites of fenpropathrin from the work of Kaneko and Miyamoto (2001) and Kaneko (2010).

Crawford and Hudson (1977) studied the metabolism of a 1.5 mg/kg oral dose of benzyl-^{14}C labeled fenpropathrin in the rat. The stereochemistry of fenpropathrin was not indicated. Fifty-seven percent of the administered dose was eliminated in urine and 40% in feces within 48 h. Fenpropathrin was hydroxylated on the phenyl ring to form 4'-OH fenpropathrin and at a *trans* methyl group on the cyclopropane ring. The intact

hydroxylated esters and fenpropathrin were cleaved by carboxylesterase action and eliminated as the glucuronide of tetramethyl CPCA (2, 2, 3, 3-tetramethyl cyclopropanecarboxylic acid; CAS no. 15641-58-4), 2-OH, trimethyl CPCA (2-(hydroxymethyl)-2, 3, 3-trimethyl-cyclopropanecarboxylic acid; CAS no. 97280-65-4) and the O-sulfates of the oxidized 3-phenoxybenzene moiety.

For ^{14}C-acid- and ^{14}C-alcohol labeled fenpropathrin, Kaneko et al. (1987) reported that 58-70% and 54-71%, respectively, of an administered dose (2.4-26.8 mg·kg^{-1}) was recovered in feces and 27-44% and 26-43%, respectively, in urine. Fenpropathrin *per se* was readily hydrolyzed by carboxylesterases or hydroxylated at the methyl group of the acid moiety and at the 4'-position of the alcohol prior to hydrolysis. The sulfate of PB acid was the major urinary metabolite along with the glucuronides 2, 2, 3, 3-tetramethylcyclopropanecarboxylic acid (TMPA) and its hydroxymethyl derivatives. Intact esters were the major fecal metabolites with hydroxylation at the 4'-position of the alcohol and the *trans*-methyl group of the acid.

5.9 Fluvalinate

Table D9, Appendix D, gives the chemical structures of the parent pyrethroid and metabolites of fluvalinate (CAS no. 69409-94-5) (Kaneko and Miyamoto 2001; Kaneko 2010). Quistad et al. (1983) prepared (αRS, 2R)-[benzyl-U-ring-^{14}C] tau-fluvalinate (CAS no. 102851-06-9) to study the fate of the alcohol moiety. Rats were given single oral doses (0.7 and 60 mg/kg) by gavage in corn oil. Previous studies by Quistad et al.

(1983) involved the administration of ^{14}CF labeled (αR, 2R), (αS, 2S), (αR, 2S) and (αS, 2R) isomers (23, 25, 28, and 24%, respectively) of fluvalinate (CAS no. 69409-94-5) (1 mg/kg) in corn oil. Urinary metabolites from the ring-labeled study of Quistad et al. (1983) included PB acid (3.9%), 4'-OH BP acid (2.3%), PB acid glycine (9.0%), and 4'-OH PB acid sulfate (72.0%). The metabolites analyzed in 1-d urine samples represented 57.0% of the single oral dose, while fecal extracts contained 75.9% fluvalinate (33.4% of administered dose), with lesser amounts of PB acid (3.1%), PB alcohol (4.0%) and 4'-OH PB acid (1.7%). No conjugates of PB acid (cholic, CAS no. 82186-87-6), (taurocholic, CAS no. 82186-90-1) or (taurochenodeoxycholic acids, CAS no. 82186-91-2) were found in feces. The presence of the 4'-OH derivative of fluvalinate was not detected in feces or urine as was shown for other pyrethroids, suggesting that hydroxylation of the phenoxy ring occurred after cleavage.

Quistad et al. (1983), studied the elimination of ^{14}CF labeled fluvalinate, and discovered that the hydroxy acid (CAS no. 82186-86-5), anilino acid (CAS no. 76338-73-3), glycine conjugate of the anilino acid, haloaniline (CAS no. 39885-50-2) and the sulfate of hydroxyhaloaniline (CAS no. 84960-10-1) were excreted in urine. Fluvalinate, hydroxyacid, anilino acid and anilino acid conjugates were found in feces. According to Quistad et al. (1983), the ratio of fecal to urinary elimination is higher for fluvalinate than for fenvalerate (Kaneko et al. 1981b; Ohkawa et al. 1979), deltamethrin (Cole et al. 1982; Ruzo et al. 1978), cypermethrin (Cole et al. 1982) and tralomethrin (Cole et al. 1982).

5.10 Permethrin

Table D10, Appendix D, gives the physical/chemical and partition coefficient data for *trans*- and *cis*- permethrin (chirality not shown) and metabolites. See Tables 2 and D10 for the structures and technical names of these. According to Michael-Rocky Goldsmith (EPA, personal communication), chirality does not change the values log P/D. The metabolites were taken from Fig. 58.17, the metabolic pathways for permethrin, as reported by Kaneko and Miyamoto (2001) and from Fig. 76.19 of the Kaneko (2010) study. The pathway takes into consideration differences in the carboxylesterase catalyzed hydrolysis rates of *trans*- and *cis*-permethrin, with more hydrolysis products being formed from the *trans* isomer.

Permethrin (CAS no. 52645-53-1) is a mixture of the (1RS, *trans*) and (1RS, *cis*) isomers. Gaughan et al. (1977) studied the metabolism of (1R, *trans*)-, (1RS, *trans*)-, (1R, *cis*)-, and (1RS, *cis*)-permethrin administered orally to rats at dosages of 1.6 to 4.8 $mg \cdot kg^{-1}$. In their study, labeled permethrins and metabolites (1-10) were used: (1R, *trans*; 1R, *cis*) permethrin labeled in the acid ($Cl_2C^*=CH$) moiety (metabolites 1,3); (1R, *trans*; 1R, *cis*) permethrin uniformly labeled (UL) in the phenoxy ring (metabolites 2, 4); (1RS, *trans*; 1RS, *cis*) permethrin labeled in the acid moiety (C*=O)(metabolites 5,7), and (1RS, *trans*; 1RS, *cis*) and permethrin labeled in the α-OH carbon (metabolites 6, 8). Two metabolites were radiolabeled, one in the acid moiety (^{14}C)-CPCA (metabolite 9) and one in the alcohol moiety [(^{14}C)-PB alcohol] (metabolite 10). The (1R, *trans*) permethrin and (1R, *cis*) permethrin are each single isomers, while the (1RS, *trans*) permethrin and (1RS, *cis*) permethrin are each mixtures of two isomers.

Male rats were individually administered the 10 compounds, with the rats administered carbonyl-labeled 5, 7)(1RS, *trans*; 1RS, *cis*) and phenoxy-labeled 2, 4)(1RS, *trans*; 1RS, *cis*) permethrin being placed in all glass metabolism cages for up to 12 d, which permitted the complete collection of urine, feces and carbon dioxide (Gaughan et al., 1977). The other six test groups were housed in metal cages for the same period of time. The metabolites retaining the ester linkage were compared by TLC to those from the administration of (1R, *trans*) permethrin, labeled in the acid (^{14}C)-DCCA and (^{14}C)-PB alcohol moieties. Products retaining the ester linkage were hydrolyzed and the degradation products recovered and identified. Conjugates were cleaved by enzyme, acid and base, and then were chromatographed.

Lactonization occurred during treatment with strong acids or as artifacts of TLC separations. The glucuronides were partially converted to lactone derivatives when dilute methanolic HCL solutions were evaporated to dryness. Ninety-seven to 100% of the two mixed *cis*-permethrin isomers and the two mixed *trans* isomers (^{14}C-acid and alcohol labeled) were recovered in urine and feces. The hydrolysis products of (^{14}C-acid-1R, *trans*) and (^{14}C-alcohol-1R, *trans*) permethrin were largely eliminated in urine (81-90%), while only 45-54% from *cis*-permethrin appeared in urine. The glucuronide of Cl_2CA was the principal metabolite found in the urines of (1R, *trans*; 56.1%) and (1RS, *trans*; 41.9%) permethrin-treated rats, while a lesser amount were found in the urines of (1R, *cis*; 18.5%) and (1RS, *cis*; 13.8%) permethrin-administered rats. For rats administered the ^{14}C-alcohol labeled permethrin, the principal metabolite was 4-OH PB acid sulfate in

urine of rats administered (1R, *trans*; 30.7%) and (1RS, *trans*; 42.8%) permethrin. Less of this metabolite was found in urine of rats administered the (1R, *cis*; 19.5%) and (1RS, *cis*; 29.3%) permethrin.

In summary, the five principal sites of metabolism on permethrin are ester cleavage, oxidation at the *trans* or *cis* methyl group of the geminal dimethyl moiety, and oxidation at the 2' or 4'-position of the phenoxy group. Considerable attention was paid by Gaughan et al. (1977) to the oxidation of the geminal dimethyl moieties; they found only a small percentage (0.3 to 1.4%) of the *trans*-methyl groups were oxidized, with an equivalent amount of oxidation (1.5-4.7%) on the *cis*-methyl group.

The importance of the oxidation of the geminal dimethyl moieties appears to disappear in some of the later studies with pyrethroids. The reason for this may be difficulties in obtaining appropriate synthesized standards and setting up good chromatographic procedures for identifying such compounds in urine. The alternative explanation is that hydroxylation of the methyl group does not occur to any extent in the other pyrethroids.

According to Anadon et al. (1996), the oral bioavailability of permethrin in the rat was 60.69%. Permethrin was administered orally and intravenously at dosages of 460 and 46 $mg \cdot kg^{-1}$ to male Sprague-Dawley rats. The maximum amount of permethrin found respectively in cerebellum, hippocampus, caudate putamen, frontal cortex, hypothalamus and sciatic nerve were respectively about 1.5, 2.2, 2.7, 4.8 and 7.5 times higher than in plasma. Certain metabolites of permethrin, PB alcohol and PB acid were detected in plasma and selected tissues 48 h after dosing.

Choi and Soderlund (2006) identified human alcohol (ADH) and aldehyde dehydrogenases (ALDH) as the enzymes involved in the oxidation of PB alcohol (phenoxybenzyl alcohol) from *trans*-permethrin to PB acid (phenoxybenzoic acid) via phenoxybenzaldehyde. *Cis*-Permethrin was not metabolized to any extent in human liver fractions. Cytochrome P450 isoforms were not involved either in the hydrolysis of *trans*-permethrin or in the oxidation of the PB alcohol to the PB acid.

5.11 Phenothrin

Table D11, Appendix D, gives the metabolites for *cis*- and *trans*-phenothrin as presented in Fig. 58.19 and 76.20, respectively, from Kaneko and Miyamoto (2001) and Kaneko (2010). The structure and technical name for phenothrin is presented in Table 2.

The chiral structures (stereochemistry) are not shown in Table D11. The *trans*-isomers are more readily hydrolyzed than are the *cis*-isomers by carboxylesterases, and are perhaps more readily hydroxylated by P450 isozymes. Kaneko et al. (1984) studied the metabolism of six isomer preparations [(1R-trans), (1RS-trans), (1S-trans), (1R-cis), (1RS-cis) and (1S-cis)] of phenothrin after single oral administration of each ^{14}C-labeled isomer or isomer mixture at 10 mg·kg^{-1} to rats and mice. There was no significant difference between the elimination of the (1R-*trans*) and (1RS-*trans*) or the (1R-*cis*) and (1RS-*cis)* isomers. However, the (1S-*trans*) and (1S-*cis*) isomers were eliminated to a

greater extent in urine than were the (1R-) and (1RS-) *trans*- and *cis*- isomers, respectively (Izumi et al. 1984).

The alcohol moiety of (1R-*trans*)- and (1R-*cis*)-phenothrin is rapidly metabolized and eliminated, with the major excreted metabolite being either free or conjugated 4-OH PB acid (CAS no. 35065-12-4) (Miyamoto et al. 1974; Miyamoto 1976). Intact esters of (1R, *cis*) phenothrin were excreted in feces, one being 4-OH phenothrin (CAS no. NA), another carboxy phenothrin (CAS no. 79897-38-4) and carboxy 4'-OH phenothrin (CAS no. 79861-56-6). Oxidation of one of the geminal dimethyl groups has also been reported (Miyamoto 1976). According to Elliott et al. (1976), phenothrin is metabolized like permethrin except for alterations on the isobutenyl group not seen for the dichlorovinyl side chain of permethrin.

5.12 Resmethrin

Table D12, Appendix D, gives the metabolites for *trans*- and *cis*-resmethrin. The metabolism of *trans*- and *cis*-resmethrin was presented by Kaneko and Miyamoto (2001) and Kaneko (2010), respectively in Fig. 58.22 and 76.23 of their publications. See Table 2 for resmethrin's structure and technical name. Bioresmethrin and cismethrin are (1R)-*trans* and (1R)-*cis* isomers of resmethrin, respectively.

Miyamoto et al. (1971) and Ueda et al. (1975) studied the rat metabolism of (1RS-*trans*), (1R-*trans*), or (1R-*cis*)-resmethrin that was individually labeled with ^{14}C at the carbonyl group of the acid moiety or the phenyl group of the alcohol moiety. The purity of the *trans*-*cis* isomers was determined by gas chromatography to be > 99.5%. Authentic

standards of unlabeled (-)-*cis*-chrysanthemic acid and 5-(phenylmethyl)-3-furanmethanol (CAS no. 20416-09-5) were used for tentative identification of resmethrin metabolites. Five additional compounds, BF acid (CAS no. 24313-22-2), *α*-keto BF acid (CAS no. 37744-71-1), α-OH BF acid (CAS no. 37744-70-0), 4-methoxy BF alcohol and 4-methoxy BF acid, were prepared according to the methods of Miyamoto et al. (1971). In reviews authored by Ueda et al. (1975) and Miyamoto (1976), it was reported that (+)-*trans*-resmethrin is readily hydrolyzed by carboxylesterases and eliminated as oxidized products of 5-(phenylmethyl)-3-furanmethanol (BF alcohol) and CPCA The oxidative degradation of (+)-cis-resmethrin by cytochrome P450 was also reported (Suzuki and Miyamoto 1974; Ueda et al. 1975) to produce geometric isomers (E- and Z-isomers) of chrysanthemic acid products (i.e., alcohol, aldehyde and acid). The proposed metabolic pathways indicate that intact resmethrin is not oxidized (hydroxylated) on one of the phenyl or furan rings of 5-(phenylmethyl)-3-furanmethanol, or at the isobutenyl methyl group, prior to hydrolysis. Table D12, Appendix D, shows the metabolic pathway for resmethrin as it was proposed by Kaneko and Miyamoto (2001), and as it was revised by Kaneko (2010). These pathways are similar, except that resmethrin *per se* in Table D12 was hydroxylated at the 4'-position of the phenyl and furan ring, and at the phenylmethyl group, before hydrolysis. Hydrolysis of resmethrin and these three resmethrin derivatives by carboxylesterases, followed by the oxidation of their alcohol leaving groups would explain the presence of their respective acids in urine. This begs the question as to why one of the methyls of the isobutenyl or gem-dimethyl group in the intact ester is not oxidized to a carboxylic acid, or to an alcohol, as shown for phenothrin prior to hydrolysis. The reason may be the rapid hydrolysis of the *trans* isomer, but this does not

explain why these groups are not oxidized by cytochrome P450s prior to hydrolysis as is the case of the *cis* isomer. Bioresmethrin (1R-*trans*) was hydrolyzed more rapidly by the carboxylesterases in rat liver microsomes (RLM) than cismethrin (1R-*cis*). The carboxylesterases in plasma did not show this *cis*-*trans* relationship (White et al. 1976).

5.13 Tefluthrin

Table D13, Appendix D, gives the metabolites of the two cis isomers of tefluthrin (PP993), (1R, 3R) and (1S, 3S) tefluthrin (CAS no. 79538-32-2 rel) (Prout et al. 1985a,b and 1986). In the Prout et al. (1985a) studies, oral doses of 1.0 mg/kg of [^{14}C]-cyclopropane ring (acid)-labeled tefluthrin and [^{14}C]-alcohol labeled tefluthrin were individually administered in corn oil to four male and four female Alpk:APfSD strain rats. The rats were individually housed in metabolism cages over a period of 7 d to collect daily urine and feces. Tissues and carcasses were harvested at the end of the 7-d period for radioanalysis. One additional male and female rat each was administered 1.0 mg/kg of [^{14}C] alcohol-labeled tefluthrin for collection of respiratory $^{14}CO_2$ over a 48-h period. The animals were then terminated and embedded in paraffin blocks for whole body autoradiography. A higher percentage of the dose was excreted in feces (53.9 to 66.6%) than in urine (20.1 to 33.4%). There were no pronounced differences in the excretion profiles of the alcohol or acid ^{14}C-labeled tefluthrin. Negligible amounts of radioactivity were eliminated as respiratory $^{14}CO_2$. In the autoradiograph, the radioactivity was located largely in the gastrointestinal tract, liver and brown fat.

In a second study by Prout et al. (1985b), the tissue distribution and excretion of [^{14}C]-alcohol labeled tefluthrin was followed after the oral administration of 10 mg/kg to four male and four female rats in corn oil. The animals were individually housed in metabolism cages for daily collections of urine and feces over a 7-d period. Rats were terminated at the end of 7 d and individual tissues harvested for the determination of radioactivity. A further set of two males and two females were administered an oral dose of 1.0 mg/kg [^{14}C] alcohol-labeled tefluthrin, after bile duct cannulation. Urine, bile and feces were collected for a period of 48 h. Signs of acute toxicity were observed in the 10 mg/kg rats. Males excreted slightly less in urine (26 vs 33%) and more in feces (68 vs. 63%) than females. In the bile duct study, males excreted 5 to 16% and females 8 to 10% of total radioactivity.

In the third Prout et al. (1986) study, urinary metabolites from tefluthrin ([^{14}C]-acid or -alcohol labeled) orally administered to rats at 1.0 mg/kg or [^{14}C]-alcohol labeled at 10 mg/kg were isolated by solvent extraction. The extracts were chromatographed by a combination of TLC, HPLC and GLC techniques to identify metabolites. The glucuronide conjugates were hydrolyzed using glucuronidase or 6M HCL at 100^{o}C. The metabolites were identified by mass spectroscopy and by co-chromatography with reference standards. The [^{14}C]-alcohol metabolites were identified as the glucuronides of tetrafluoro-1, 4-benzenediol (CAS no. 142209-31-2), and tefluthrin alcohol, 18 to 37% and 13 to 28%, respectively. Additional metabolites in urine were 10 to 18% tetrafluoro-4-OH methyl benzoic acid (CAS no.107900-84-5), and 12-28% tetrafluoro-4-methyl-benzoic acid (CAS no. 652-32-4). The characterized [^{14}C]-acid-

tefluthrin metabolites in urine were the lactone of 2-OH methyl TFP acid (CAS no. 107900-83-4) and TFP acid (CAS no. 74609-46-4) and its glucuronide (CAS no. 120851-78-7).

The bile contained the glucuronides of two metabolites, tefluthrin alcohol (CAS no. 79538-03-7) and tetrafluoro-1, 4-benzenediol (CAS no. 142209-31-2). No parent compound was found in the bile. Analysis of fecal extracts from the rats administered the [^{14}C]-alcohol label contained mostly unchanged or unabsorbed tefluthrin. Metabolites consisted of 4-OH methyl tefluthrin (CAS no. 120851-77-6), 2-OH methyl tefluthrin (CAS no. 120808-42-6) and 2-OH methyl, 4-OH methyl tefluthrin (CAS no. NA). Several minor metabolites were found, two of which were tetrafluoro-4-OH methyl benzoic acid (CAS no. 107900-84-5) and tetrafluoro-4-methyl benzoic acid (CAS no. 652-32-4).

5.14 Tetramethrin

In Table D14, Appendix D, the metabolites of *trans*- and *cis*-tetramethrin are given. The metabolic pathway in this table was taken from the work of Kaneko and Miyamoto (2001) and Kaneko (2010), Fig. 58.23 and 76.24, respectively. Tetramethrin is a 4:1:1:4 mixture of four isomers with the two *trans* isomers (1R, 3R) and (1S, 3S) being more abundance than the two *cis* isomers. Only the metabolism of the (1R, 3R) and (1R, 3S) isomers were studied by Kaneko et al. (1981c). The oxidized and sulfonic acid intermediates were not shown in the Kaneko and Miyamoto (2001) review, were added to Table D14.

Kaneko et al. (1981c) identified the metabolites of ^{14}C carboxyl-, and alcohol-labeled (1R)-*trans*- (CAS no. 1166-46-7) and (1R)-*cis*-tetramethrin (CAS no. 51348-90-4) in the urine of rats. A small amount of the *cis* isomer or its ester metabolites were eliminated in feces. No *trans*- isomer or ester metabolites were found in feces. TPI (3,4,5,6-tetrahydrophthalimide; CAS no. 4720-86-9), its glucuronide (CAS no. 81951-66-8), HPI (CAS no. 1444-94-6), and its 3a-hydroxy (CAS no. 81951-68-0), 4-hydroxy (CAS no. 17605-52-6), and 5-hydroxy derivatives (CAS no. 81951-69-1), THAM (CAS no. 81951-65-7) and carbon dioxide were identified as ring metabolites of *trans*- and *cis*-tetramethrin. The chrysanthemic acid metabolites found were three dicarboxylic acids, (1R, 3R)-E isomer (CAS no. 72120-98-0), (1R, 3R)-Z isomer (CAS no. 56390-95-5), (1R, 3S)-E isomer (56390-97-7), and three hydroxymethyl monoacids, (1R, 3R)-E isomer (CAS no. 54324-84-4), (1R, 3S)-E isomer (CAS no. 54276-09-4) and (1R, 3S)-Z isomer (CAS no. 56390-96-6).

Tomigahara et al. (1994b) administered ^{14}C-alcohol-labeled (1RS)-*trans* or (1RS)-*cis* tetramethrin to rats orally at doses of 2 and 250 mg/kg. In 7 d, 38-56 and 42-58% of the ^{14}C *trans*- isomer was recovered in feces and urine, respectively. These same values for the *cis* isomer were 66-91 and 9-31% of the ^{14}C in feces and urine, respectively. In feces, the main metabolites were sulfonate derivatives, and in urine, the alcohol and dicarboxylic acid derivatives from the 3,4,5,6-tetrahydrophthalimide moiety. Two of the five sulfonic acids (*trans*-acid-3-OH-NPY-SA and TPI-SA) were detected in urine. The

sulfonic acids were believed to have been formed in the intestinal tract, because the sulfonic acids were not detected in the urine of bile duct-cannulated rats.

5.15 Tralomethrin

Tralomethrin differs from deltamethrin in having 3-tetrahaloethyl substituent instead of 3-dihalovinyl groups. The 1R, αS-*cis* configuration (CAS no. 66818-66-4 and 66841-22-3) confers the highest insecticidal potency, and esters with the R and S configurations at the 1' center of the 3 side chain are comparable in activity (Ackermann et al. 1980). Tralomethrin is rapidly converted to deltamethrin in insects (Ruzo and Casida 1981). The metabolic pathway in Appendix D, Table D15, was taken from Kaneko and Miyamoto (2001; Fig. 58.24) and Kaneko (2010).

(1'S)-Tralomethrin administered orally to mice undergoes 25% debromination in the stomach within the first hour after dosing. The metabolic pathway then involves two starting products, tralomethrin and deltamethrin with deltamethrin being the final starting product. Tralomethrin is readily debrominated by tissue thiols, such as glutathione. Cole et al. (1982) orally administered ^{14}C-alcohol (benzylic methane), ^{14}C-acid (geminal dimethyl) and ^{14}C-CN (cyano)-labeled tralomethrin [(*S*)-cyano(3-phenoxyphenyl)methyl (1R,3R)-2,2-dimethyl-3-[(1S)-1,2,2,2-tetrabromoethyl]cyclopropanecarboxylate] (CAS no. 68198-90-3) individually to rats (0.32 mg·kg^{-1}) and recovered the ^{14}C-alcohol-labeled dose in urine (42.6%), feces (56.0%) and tissues (1.4%) at the end of the study. These recovery values are quite similar to those obtained using radiolabeled deltamethrin.

Metabolites from acid-^{14}C, alcohol-^{14}C and ^{14}CN labeled preparations and their percentages were identified in urine and feces by Cole et al. (1982). Products retaining ester linkage were found in feces, while hydrolysis products were present in urine. Tralomethrin is not normally detected in feces *per se* or as hydroxylated products. No X_4CA (carboxylic acid) was detected in excreta. The evolution of $^{14}CO_2$ from the ^{14}C-alcohol compounds is a minor event, as is the case for $^{14}CO_2$ from $^{14}CN^-$ (Ohkawa et al. 1979) labeled pyrethroids. Staiger and Quistad (1984) also reported on the nature of the urinary metabolites of tralomethrin resulting from the administration of 0.32 $mg \cdot kg^{-1}$ to rats. The same metabolites were eliminated by rats receiving deltamethrin (0.32 $mg \cdot kg^{-1}$) as were those receiving tralomethrin (0.32 $mg \cdot kg^{-1}$). The sulfate of 4'-OH PB acid arising from the administration of deltamethrin was higher (47.5%) than from tralomethrin (24.3%).

6 Metabolic Enzymes, Carboxylesterases and Cytochrome P450s

6.1 Carboxylesterases (CEs) and their Multiple Forms

According to Hosokawa et al. (1987, 1990), and Satoh and Hosokawa (1998), CEs are members of the α/β hydrolase family of enzymes. The family provides a stable scaffold for the active sites of the enzymes. The catalytic residues constitute a highly conserved triad consisting of a nucleophile (serine, cysteine or aspartic acid), an orientating acid, glutamate, and a histidine residue. The nucleophile is located in a sharp turn, called the "nucleophile elbow", where it is easily approached by the substrate, as well as by the

hydrolytic water molecule (Nardini and Dijkstra 1999). The α/β hydrolase fold family is the first example of enzymes with a glutamate residue. The acidic member is located in a reverse turn, usually following strand β7. The histidine residue is the site that is absolutely conserved. The loop may differ considerably in shape and length among the various members of the family.

Mammalian liver CEs are encoded by multiple genes. The isozymes were initially classified by their substrate specificities and pI (isoionic point). The two major human liver isozymes, hCE-1 and hCE-2, belong to two classes: CES1 and CES2, while rat CEs belong to two classes, hydrolases A and B. CEs may currently be classified into five major groups, CES1 to CES5, according to the homology of their amino acid sequence (Satoh and Hosokawa 1998, 2010). The CES 1 family includes the major form of CE isozymes which may be subdivided into eight subfamilies: CES1A, CES1B, CES1C, CES1D, CES1E, CES1F, CES1G and CES1H. Most of the CES1 family, except for CES1G, is expressed in the liver. The CES1A subfamily includes the major forms of human CEs. A high level of CES1 activity is present in the blood of rats and mice with no activity present in human blood. In contract to CES1, the CES2 family is mainly expressed in the small intestine. Stok et al. (2004) identified a pyrethroid-hydrolyzing carboxylesterase from mouse liver microsomes by mass spectral sequence analysis of the protein. Two peptide sequences were identical to sequences in a putative carboxylesterase (NCBI accession number BAC-36707). A third peptide also corresponded to the same putative carboxylesterase with one amino acid difference from the reported sequences. Stok et al. (2004) also found that, during the expression and purification of

carboxylesterase, the protein was not as closely associated with the microsomes; 63% was found in the cytosol and only 11% in the microsomes.

Analysis of human CE by Northern blot shows a single band of approximately 2.1 kilobases (kb)(Riddles et al. 1991), and three bands of approximately 2, 3 and 4.2-kb occurring with hCE-2 (Schwer et al. 1997). The intensities of the 2.1-kb band were liver >> heart > stomach > testis >= kidney = spleen > colon > other tissues. For hCE-2, the 2-kb band was located in liver > colon > small intestine > heart >, the 3-kb band in liver > small intestine > colon > heart, and the 4.2-kb band in brain, testis, and kidney only. Analysis of substrate structure versus efficiency for ester (pyrethroid substrates) revealed that the two CEs recognize different structural features of the substrate (i.e., acid, alcohol, etc.). The catalytic mechanism involves the formation of an acyl-enzyme on an active serine. While earlier studies of pyrethroid metabolism were primarily performed in rodents, knowledge of the substrate structure-activity relationships and the tissue distribution of hCEs are critical for predicting the metabolism and pharmacokinetics of pesticides in humans. Wheelock et al. (2003) used a chiral mixture of the fluorescent substrate cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, cyano(6-methoxy-2-naphthalenyl)methyl ester (CAS No. 395645-12-2) to study the hydrolytic activity of human liver microsomes. Microsomal activity against this substrate was considered to be low (average value of 10 samples: 2.04 ± 0.68 nmol min^{-1} mg^{-1}), when compared to p-nitrophenyl acetate (CAS No. 830-03-5) at 3700 ± 2100 nmol min^{-1} mg^{-1}.

6.2 Cytochrome P450s and their Multiple Forms

CYP450 was first shown to be a hemoprotein by Omura and Sato (1964). It has a noncovalently bound iron protoporphyrin IX prosthetic group, similar to the heme in b-type cytochromes, hemoglobin, and myoglobin. The heme prosthetic groups may be removed from the protein by acidic acetone, alkali, or pyridine. The binding of carbon monoxide provides a means for their analysis and quantification. The enzymes of the CYP-dependent monoxygenase system are embedded in the endoplasmic reticulum of liver hepatocytes and the cells of other organs. The endoplasmic reticulum is harvested from a cell homogenate by centrifugation at 16,000 g to remove large particles and then at 105,000 g to pelletize the microsomes (endoplasmic reticulum) from the supernatant (Lu and West 1980). The P450 enzymes are distributed between the two surfaces of the microsomal bilayer with the NADPH-CYP reductase and P450 facing the cytoplasm of the cell. Therefore, the monoxygenase system is composed of three components: reductase protein, a lipid fraction, and CYP protein. The purification of rat and human liver P450s is described by Guengerich and Martin (1998). Estimation of protein concentrations provides a means for evaluating the progress of purification. The content of CYP heme should be about 18 $nmol \cdot mg^{-1}$ protein.

Multiple forms of CYPs exist in liver microsomes. These forms play a role in the oxidation (i.e., hydroxylation of aromatic, aliphatic, and alkyl groupings) of pyrethroid insecticides. The major isoforms in human liver include CYP 1A2, 2A6, 2B6, 2C8, 2C19, 2E1, 3A4, 3A5, and 3A7 (fetal livers). There are large individual variations in the microsomal content of these forms in human liver. A calculator for determining the content of these P450 isozymes in human liver (ages 1-18 yr) was presented by Foxenberg et al. (2007). The recombinant human CYPs are available from a Baculovirus-

insect cell expression system along with the coexpression of NADPH-CYP reductase (Supersomes; Gentest, Woburn, MA). Yields range from 50 to 250 pmol P450 mg^{-1} microsomal protein as determined by CO difference spectral analysis (Hood et al. 1998). The major isoforms in rat liver include CYP1A1, 1A2; 2A1; 2B1; 2C6, 11, 12, 13; 2D1, 2D2; 3A1, and 3A2 (Supersomes, BD Biosciences (Woburn, MA)). It is important to note that human and rat CYPs exhibit significant differences in protein structure as well as catalytic activity. Thus, there is a critical need to conduct future studies of pyrethroid metabolism with human CYPs and CEs, since most researchers who have published in the literature have utilized rodent models.

6.3 Hydrolysis of Pyrethroids by CEs

Ross et al. (2006) studied the hydrolytic metabolism of Type I pyrethroids (bioresmethrin, 1RS *trans*-permethrin, and 1RS *cis*-permethrin) and several Type II pyrethroids (alpha-cypermethrin and deltamethrin) by pure human CEs (hCE-1 and hCE-2), a rabbit CE (rCE), and two rat CEs (Hydrolases A and B). Hydrolysis rates were based on the formation of 3-phenoxybenzyl alcohol (PBAlc) (CAS no. 13826-36-2) for the cis and trans isomers of permethrin. For bioresmethrin, hydrolysis was based on the production of the *trans*-chrysanthemic acid (CPCA) (CAS no. 10453-89-1). For alpha-cypermethrin and deltamethrin, hydrolysis was based on the formation of *cis*-permethrinic acid (DCCA) (CAS no. 57112-16-0) and 3-phenoxybenzyl aldehyde (PBAld: CAS no. 39515-51-0), respectively. Human CE-1 and hCE-2 hydrolyzed *trans*-permethrin 8- and 28-fold more efficiently (based on k_{cat}/K_m values) than did *cis*-permethrin, respectively. The kinetic parameters (V_{max}, K_m) for the hydrolysis of *trans*-

and *cis*-permethrin, bioresmethrin and alpha-cypermethrin by rat, mouse, and human hepatic microsomes are given in Table 7. The *trans*- isomer of permethrin is more readily hydrolyzed by rat, mouse and human hepatic microsomal carboxylesterase than *cis*-permethrin (13.4, 85.5 and 56.0 times, respectively). However, the lower K_m for hydrolysis of cis-permethrin in human microsomes suggests that there are both stereoisomer and species-specific differences in metabolism kinetics.

[Insert Table 7 about here.]

The relative levels of hCE-1 protein were measured in human microsomes by immunoblotting to determine if variation in hydrolytic metabolism was related to the abundance of hCE-1 in the microsomes. Human CE-1 levels did not correlate ($r = 0.294$) well with V_{max} values for the hydrolysis of *trans*-permethrin. Esterases other than hCE-1 are believed to be responsible for the different V_{max} values. CDMB (1,2-ethanedione, 1-(2-chlorophenyl)-2-(3,4-dimethoxyphenyl)-(CAS no. 56159-70-7), was used to inhibit the activity of hCE-2 (Wadkins et al. 2005). The results suggested that a significant portion of the microsomal activity was catalyzed by hCE-2. An anti-body was not available for detecting or measuring hCE-2. Turnover numbers (k_{cat}/K_m, $mM^{-1}\cdot min^{-1}$) indicate that *trans*-permethrin is as efficiently hydrolyzed by hCE-1 as it is for hCE-2. Bioresmethrin is not hydrolyzed by hCE-2 suggesting that metabolism is primarily mediated by hCE-1. The rates of hydrolytic metabolism by rabbit carboxylesterase followed the order: bioresmethrin > *trans*-permethrin > *cis*-permethrin > deltamethrin >

alpha-cypermethrin. Ross et al. (2006) did not extrapolate hydrolytic in vitro V_{max} values (nmol·min^{-1}·mg^{-1} of microsomal protein) into in vivo values (μmol·hr^{-1} ·kg^{-1} of body weight (bwt)) for use in PBPK models.

Crow et al. (2007) examined the catalytic activity of carboxylesterase (rCE proteins: hCE-1 and hCE-2) on Type I pyrethroids (i.e., 1RS *trans*-permethrin, (98% pure, 93% *trans* and 5% *cis*), 1RS *cis*-permethrin (99% pure), 1R *trans*-resmethrin (bioresmethrin, 99% pure, 97% *trans* and 2% *cis*); Type II pyrethroids (i.e., alpha-cypermethrin (99% pure, mixture of isomers), lambda-cyhalothrin (99% pure, mixture of isomers) and deltamethrin (99% pure).

Human intestinal microsomes effectively hydrolyzed *trans*-permethrin; however, bioresmethrin and deltamethrin were not metabolized in the intestine to any appreciable extent. Human hepatic microsomes and cytosol contained both hCE-1 and hCE-2 when examined by native PAGE (polyacrylamide gel electrophoresis); human intestine contained only hCE-2. Table 8 gives the kinetic parameters obtained by Crow et al. (2007) with *trans*-permethrin and liver and intestinal carboxylesterases.

[Insert Table 8 about here.]

Rat serum was found capable of hydrolyzing the six pyrethroids studied by Crow et al. (2007), with bioresmethrin and *trans*-permethrin being hydrolyzed the fastest and *cis*-permethrin, deltamethrin and esfenvalerate the slowest. Humans lack detectable amounts of CEs in blood. On this basis, the rat should be considered a poor surrogate animal for

human health risk assessment at the environmentally low levels at which the pyrethroids normally exist (Crow et al. 2007).

6.4 In Vitro Metabolism

Scollon et al. (2009) studied the in vitro metabolism of bifenthrin (100% *cis*), S-bioallethrin (100% *trans*), bioresmethrin (96% *trans*/2% *cis*), β-cyfluthrin (67% *trans*/33% *cis*), γ-cyhalothrin (100% *cis*), cypermethrin (49% *cis*/51% *trans*), *cis*-permethrin (100% *cis*), *trans*-permethrin (96% *trans*/4% *cis*), and resmethrin (30% *cis*/70% *trans*) by rat and human liver microsomes and cytochrome P450 isozymes. Rat liver microsomes (Long-Evans males, 275-299 g) were prepared from a pool of livers from six animals. Three samples of mixed gender pool human adult liver microsomes were purchase from three different sources (CellzDirect, Phoenix, AZ; Cedra, Austin, TX; and XenoTech, LLC, Lenexa, KS). Each pool was used in a separate study. Assays were conducted by measuring the total disappearance of parent compounds over 10 min with and without NADPH. Pyrethroids were added in 25 μL of methanol at time 0 to obtain incubation concentrations of 0.1, 1.5, 10, 20, or 50 μM. Aliquots were removed at 0, 2, 6, 8, and 10 min for analysis using HPLC/tandem mass spectrometry and the results converted to moles of substrate remaining. Substrate remaining was converted to product formed and plotted versus time to produce a reaction velocity. Reaction velocities were plotted against initial concentrations to yield estimates of K_m and V_{max} (JMP version 6; SAS Institute, Cary, NC) for each pyrethroid. The results are given in Table 9.

[Insert Table 9 about here.]

The metabolism of bifenthrin, S-bioallethrin, and *cis*-permethrin in rat and human hepatic microsomes was the result of oxidative processes, while the metabolism of bioresmethrin and cypermethrin in human hepatic microsomes was hydrolytic (not shown in Table 8). Cypermethrin and bioresmethrin were metabolized by oxidation and hydrolysis in rat hepatic microsomes. *Trans*-permethrin and β-cyfluthrin were metabolized by both pathways in human and rat hepatic microsomes.

In addition to the microsomal studies, Scollon et al. (2009) purchased rat and human Supersomes (containing P450 reductase) from BD Biosciences (Woburn, MA) to use in pyrethroid metabolism studies. The individual Supersomes contained rat CYP1A1, 1A2; 2A1; 2B1; 2C6, 11, 12, 13; 2D1, 2D2; 3A1, 3A2 and human CYP1A1, 1A2; 2B6; 2C8, 2C9*1, 2C9*2, 2C9*3, 2C19; and 3A4 P450 isoforms. The P450 content ranged from 1000 to 2000 pmol/mL. Nine pyrethroids (six of Type I and three of Type II) were screened for metabolism with or without NADPH in the incubation medium. The rat isoforms that showed activity were CYP1A1 (9/9 pyrethroids), 1A2 (3/9), 2C6 (9/9), 2C11 (8/9), 2D1 (1/9), 3A1 (8/9), and 3A2 (6/9), with human isoforms CYP1A1 (1/9 pyrethroids), 1A2 (1/9), 2B6 (1/9), 2C8 (3/9), 2C9*1 (4/9), 2C9*2 (2/9), 2C19 (9/9), and 3A4 (2/9) showing activity. The percentage of each parent pyrethroid metabolized during a 10 min incubation period was presented, while no isoform V_{max}, or K_m values were determined in the study. The results suggest that human CYP2C19 is the most important mediator of CYP-dependent metabolism and highlights the need to assess species-and CYP-isozyme specific metabolism of pyrethroids.

Godin et al. (2006) used an Agilent 1100 series LC/MSD VL ion trap mass spectrometer in their in vitro studies involving the metabolism of deltamethrin and esfenvalerate by human and rat microsomes and purified carboxylesterases. Pyrethroids were 98%+ pure; deltamethrin was obtained from Bayer Crop Sciences (Research Triangle Park, NC) and esfenvalerate from Dupont (Johnston, IA). The kinetics of hydrolysis of deltamethrin by hCE-1 (human) and hydrolase A (rat) were measured based on the release of PBAld (CAS no. 39515-51-0), which is spontaneously formed from the cyanohydrin (Wheelock et al. 2003). The metabolism of deltamethrin and esfenvalerate from rat liver microsomes occurred primarily via NADPH-dependent pathways (P450s). The independent-NADPH pathway was 20% in the case of deltamethrin and 11% for esfenvalerate. TEPP (diphosphoric acid, P, P, P', P'-tetraethyl ester, CAS no. 107-49-3) was used to inhibit the NADPH independent hydrolytic pathway catalyzed by carboxylesterases. In contrast to the rat, human liver microsomes metabolize deltamethrin by the NADPH-independent hydrolytic pathway, while estenvalerate was metabolized by the CYP-dependent pathway. The high level of hydrolysis of deltamethrin reported in Tables 10 and 11 (particularly for hCE-1) appear to be consistent with the human k_{cat} values reported in Table 11 and Figs. 11 and 12.

[Insert Tables 10-11 about here.]

[Insert Figs. 11 & 12 about here.]

In their work to extrapolate the in vitro clearance to in vivo clearance (Cl_{int}) on a per kilogram basis, Godin et al. (2006) assumed humans and rats have 40 and 25.7 g of liver per kg of body weight, respectively (Davies and Morris 1993). The microsomal content of the livers was assumed to be 52.5 and 45 mg of microsomal protein per g of liver for humans (Iwatsubo et al. 1997) and rats (Houston 1994), respectively.

In another study by Godin et al. (2007), the rat and human CYP450 isoforms and rat serum esterase that metabolized deltamethrin and esfenvalerate were identified. Table 12 gives the V_{max} and K_m kinetic parameters for the metabolism of deltamethrin and esfenvalerate by rat and human recombinant cytochrome P450s.

[Insert Table 12 about here.]

Rat serum hydrolyzed deltamethrin and esfenvalerate at rates of 15.23 ± 3.24 and 9.97 ± 2.94 $pmol \cdot min^{-1} \cdot ml^{-1}$ of serum, respectively. Neither deltamethrin nor esfenvalerate were hydrolyzed when incubated with human sera, supporting the lack of CE activity in human blood.

6.5 Binding of Substrates to Microsomes and CYPs; Prediction of K_m and V_{max}

In most in vitro microsomal metabolism studies, the concentration of microsomal protein used in incubation media is approximately 1.0 $mg \cdot ml^{-1}$, whereas, substrate concentrations range from 1.0 to 100 μM, depending upon their solubilities. In such studies the concentration of free or unbound substrate is rarely measured. Obach (1997) showed that increases in microsomal protein over one or more substrate concentrations, resulted in a change in free or unbound substrate. These results suggest that the values obtained for K_m should be adjusted for the concentration of free or unbound substrate.

Unbound or free drug is determined by an equilibrium dialysis procedure. Obach (1997) compared the values obtained by incorporating the rates of microsomal binding with those in which this factor was ignored. In most cases clearance (V_{max}/K_m ratio) was lower when binding was ignored. Austin et al. (2002) published the following equation for clearance:

$$CL = \frac{\frac{Q * A * B * fu_p * CL_{int}}{fu_{inc}}}{(Q + \frac{A * B * fu_p * CL_{int}}{fu_{inc}})} \quad [27]$$

where A is a constant representing the mg of microsomes per gram of liver, B is a constant describing the grams of liver per kg of body weight, Q represents liver blood flow, fu_p is the free fraction of the compound in plasma, and fu_{inc} is the free fraction of the compound in the microsomal incubation.

In Eq. [27], a value for fu_{inc} is needed and may be determined using Eq. [28]

$$fu_{inc} = \frac{1}{C * 10^{0.56 \log P/D - 1.41} + 1} \quad [28]$$

where C is the microsomal protein concentration in mg per ml, log P/D is the log P of the molecule if it is a base (basic pKa >7.4), and log P/D is the log $D_{7.4}$ of the molecule if it is neutral or an acid (acidic pKa <7.4) (Austin et al. 2002; Hallifax and Houston 2006). In a microsomal binding study carried out with amitriptyline (CAS no. 50-48-6), binding was discovered to increase with increasing microsomal protein concentration and was drug-concentration independent over the range of concentrations used in vitro (Venkatakrishnan et al. 2000). Binding produces an over estimation of K_m without affecting the metabolic rate at concentrations that saturate the enzyme. Correcting for microsomal binding, by using the free fraction in incubation matrices, improves the prediction of in vivo clearance from in vitro estimates of clearance for drugs that are extensively bound (Obach 1996, 1997, 1999).

Because a majority of the in vitro work is now being carried out with rCYPs, the question is whether we should be concerned about nonspecific binding that occurs in rCYP studies? According to Bolger (Simulations-Plus, Inc.), the range of protein concentrations used in human liver microsome incubations lies between 3.2 $\mu g \cdot ml^{-1}$ and up to 1.0 $mg \cdot ml^{-1}$, while the range in rCYPs range from 1.25 $\mu g \cdot ml^{-1}$ up to 1 $mg \cdot ml^{-1}$, suggesting that correction may be needed for CYP studies, as well. On the basis of K_m data plots developed by Youdim and Dodia (2010), which permits one to compare the K_m values from rCYPs vs. HLM for product formation and substrate depletion, the correction on K_m was found to be less for rCYPs than for HLMs (Bolger 2010). Youdim and Dodia (2010) recommended correcting K_m by multiplying it by the substrate fraction unbound in microsomes, f_u (microsomes), as determined by equilibrium dialysis. The calculated

results for fu_{inc} in Eq. [28] may also be used. Metabolism in the liver is then defined by Eq. [29]:

$$R_{Metabolism} = \sum_{i=1}^{n} \frac{V_{\max,i} C_{liv}}{[(K_{m,i} * f_{u(microsomes)}) + C_{liv}]} \qquad [29]$$

where $V_{max,\,i}$ is the maximum velocity constant of the *i*th metabolic enzyme, $K_{a,i}$ is the corresponding concentration at which half the maximum velocity is reached; f_u (microsomes) is the fraction of the drug unbound in microsomes; and n is the number of enzymes involved in clearing the drug.

To our knowledge, a corrected K_m value has not been utilized in the development of Michaelis-Menten constants for the pyrethroid insecticides.

7 QSAR Models for Predicting Biological Parameters Used in PBPK/PD Models

7.1 Toxicity Models

TOPKAT (Enslein et al. 1998) is a toxicity model (for estimating LD_{50}, etc.) and was among the first QSAR models to be developed for predicting the toxicity of pesticides and other chemicals. Software packages, such as DEREK (LHASA, Leeds, UK), CASE and MULTICASE (Multicase, Cleveland, OH), COMPACT, HazardExpert (Compudrun, Budapest, Hungary), ONCOLOGIC (Logichem, Boyertown, PA), and ACD/Tox Suite (Advance Chemistry Development, Inc. Toronto, Canada), are able to predict the toxicity

of substances based on chemical structure. ACD/Tox Suite is the latest software product to be marketed specializing in human models that involve hERG (human endoplasmic reticulum) inhibition, CYP3A4 inhibition, genotoxicity, acute toxicity (rodent LD_{50}), aquatic toxicity, health effects, irritation and endocrine system disruption.

The European Commission, under the 5th Framework Programme, supported the development of five predictive Demetra QSAR models (e.g., acute toxicity for rainbow trout, water flea, bobwhite quail and honeybees) against five end points (Porcelli et al. 2007). The models were developed to comply with OECD (Organization for Economic Cooperation and Development) principles for validating QSARs for regulatory use. The models require inputting descriptors and SMILES for pesticides of interest to obtain predicted LD_{50} values for oral toxicity or other exposure parameters. The software is written in Java™ and can run in any machine that has Java or Java 2 runtime environments. The environmental models may not directly predict pyrethroids toxicity to humans or even laboratory animals, but the approach to developing these models for humans, etc., are similar. The major drawback of the Demetra QSAR models appears to be the need to input values for descriptors/SMILES notation for the pesticide/end point of interest to predict LD_{50} values in $mg \cdot kg^{-1}$ of body weight. The only requirement should be the end point and name of the pesticide, or at most, the SMILES for that pesticide.

At the present time, no predictive QSAR models were found that describe the neurotoxicity of the pyrethroids insecticides.

7.2 QSAR Models for Predicting the Binding of Pyrethroids to Plasma and Hepatic Proteins

Pyrethroids bind strongly to plasma proteins before being distributed by the blood to the organs for metabolism and elimination. Only the unbound or free pyrethroids contribute to toxicity and are susceptible to metabolic reactions. Pyrethroids that have a high binding constant for a plasma protein show lower toxicity and delayed clearance. Human serum albumin (HSA) is the major constituent in plasma. HSA has two ligand-specific binding sites, site I binds warfarin (CAS no. 81-81-2) and site-II binds diazepam (CAS no. 439-14-5). In addition to these binding sites, the pyrethroids may bind to lipoproteins and to alpha 1-acid glycoproteins (AGP). Several quantitative structure-property (QSPR) or QSAR models have been published (Yamazaki and Kanaoka 2004; Saiakhov et al. 2000; Colmenarejo et al. 2001; Colmenarejo 2003). Yamazaki and Kanaoka used the drug data of Vozeh et al. (1990), which contained the protein binding percents of 346 drugs, Saiakhov et al. (2000) obtained protein binding affinity information from Goodman and Gilman's textbook (Goodman 2001); Colmenarejo et al. (2001) and Colmenarejo (2003) used HSA binding data from high-performance affinity chromatographic studies. QikProp (Schrodinger, LLC) used the data base of Colmenarejo et al. (2001) to develop a $LogK_{hsa}$ model for predicting $LogK_{hsa}$ values for drugs of interest. The $QPLogK_{hsa}$ model was used to determine the $LogK_{hsa}$ values for the pyrethroids of interest and their metabolites.

7.3 Intestinal Permeability

QikProp 3.0 (Schrodinger, LL) was used to predict log P Caco-2, log P MDCK values and human oral absorption in percent for the 15 pyrethroids targeted in this review. The values obtained from application of this model are given in Table 13, along with QPLog BBB values.

[Insert Table 13 about here.]

7.4 Liver CYP450 Hydroxylation Models

QSAR models (Enslein et al. 2007) for predicting microsomal and CYP 3A4 V_{max} and K_m phase I reactions involving hydroxylation of (1) drug aromatic and (2) alicyclic rings and aliphatic groups were reported by Knaak et al. (2008) in their review of parameters for carbamate models. The manuscript submitted for publication in J CompAided Mol Design was returned and the models revised and ultimately were included in Simulations-Plus, "ADMET Predictor," Section 4.14.1 Kinetic Models for Recombinant Human Cytochrome P450 Enzymes 1A2, 2C9, 2C19, 2D6, and 3A4. The numbers of compounds in the respective CYP models were 51, 45, 42, 40, and 68. The data were obtained from the open literature, were carefully screened for appropriate experiment methodology, and were assembled into data bases. Preliminary models were built with structural descriptors from Chemical Computing Group's MOE ™ and Semichems's CODESSA ™. However,

the final models exclusively used ADMET Predictor's 2D descriptors and Artificial Neutral Network Ensemble (ANNE) modeling methodology.

The models have been updated several times (Enslein, 2010). The current statistics for version 5.5 of ADMET Predictor is as follows:

Isoform:	1A2	2C9	2C19	2D6	3A4
Number of compounds	51	53	42	36	68
RMSE (root mean square error) log K_m	0.41	0.35	0.38	0.47	0.49
RMSE (root mean square error) log V_{max}	0.57	0.71	0.54	0.31	0.62

The Enslein Model in "ADMET Predictor" supplies K_m and V_{max} values, respectively, in units of μM and nmol $min^{-1}nmol^{-1}$ P450. For user convenience, V_{max} is converted into units of nmol min^{-1} mg^{-1} microsomal protein (human liver) and into metabolic intrinsic clearance units of μL min^{-1} mg^{-1} microsomal protein. The conversion factors were obtained from Inoue et al. (2006). The Enslein Metabolism Module was used to predict K_m and V_{max} values for the hydroxylation of 15 pyrethroids by CYPs 1A2, 2C9, 2C19, 2D6, and 3A4. The results are given in Tables 14-18.

[Insert Table 14 about here.]

[Insert Table 15 about here.]

[Insert Table 16 about here.]

[Insert Table 17 about here.]

[Insert Table 18 about here.]

In studies involving intact microsomes, the in vitro V_{max}, in nmol min^{-1} mg^{-1} of microsomal protein is multiplied by mg of microsomal protein g^{-1} of liver and by g of liver kg^{-1} of body weight to obtain the in vivo value. A number of the V_{max} values were out of the range of the QSAR model. No pyrethroids were used in Enslein's training sets. Pyrethroid K_m and V_{max} values may be added to the Enslein model to improve predictability. In the ADMET Predictor, V_{max} values were expressed in nmol min^{-1} $nmol^{-1}$ of rCYP. The values were also available (calculated) in nmol min^{-1} mg^{-1} of microsomal protein.

The in vitro V_{max} units for recombinantly expressed systems (rCYPs) for permethrin were converted to in vivo values based on the following equation:

$$CL_{\text{int}}(L/H) = \left[\sum_{j=1}^{n} \left(\sum_{i=1}^{n} \frac{V_{\max}(rhCYP_j)_i X_j}{K_m(rhCYP_j)_i} \right) \right] * MPPGL * Liverwt \quad [30]$$

where,

$\sum_{j=I}^{n}$ represents the summation of the number of j CYP Isoforms, $\sum_{i=I}^{n}$ represents the summation of the number i metabolic pathways, V_{max},

the rate of metabolism in pmol min^{-1} $pmol^{-1}$ $rCYP_j$ and X_j CYP abundance in the population in pmol CYPj mg^{-1} microsomal protein. Cl_{int} is the intrinsic clearance (V_{max}/K_m) and MPPGL, the amount of microsomal protein per gram of liver.

When individual CYPs act on the pyrethroid insecticides, hydroxylation may occur at more than one position on the aromatic rings and on one or more alkyl groups. The Enslein QSAR model, however, uses V_{max} and K_m values for the hydroxylation of one site on the drugs used in the training set. No pyrethroids were included in the Enslein models. The predicted pyrethroid V_{max} values represent the hydroxylation of more than one aromatic or alkyl site by the individual CYPs. Total hydroxylation represents the summation of the activity of the individual CYPs.

Rodrigues (1999) described the multiplication of V_{max}, in terms of pmol min^{-1} $pmol^{-1}$ CYP by the mean specific content of the corresponding CYP in native liver microsomes as the "normalized rate" (NR) in pmol min^{-1} mg^{-1} of microsomal protein. The normalized rate for each CYP is totaled to give total normalized rate (TNR). Each NR may be expressed as a percent of the TNR (% TNR). Foxenberg et al. (2011), using the values obtained from human rCYPs, expressed the total amount of parent OPs (organophosphate pesticides; viz., chlorpyrifos or parathion) metabolized in liver to its oxon or dearylated form (TCPy or PNP), respectively as:

$$RAM = \frac{[V_{\max A}(C)]}{[K_{mA} + C]} + \frac{[V_{\max B}(C)]}{[K_{mB} + C]} + \frac{[V_{\max C}(C)]}{[K_{mC} + C]} + \frac{[V_{\max D}(C)]}{[K_{mD} + C]} + \frac{[V_{\max E}(C)]}{[K_{mE} + S]} + \frac{[V_{\max F}(C)]}{[K_{mF} + C]} \quad [31]$$

where RAM is the rate of metabolism in pmol·h^{-1}·kg^{-1} of bwt, CYP A-F content = pmol CYP/mg of microsomal protein; CYP A-F activity = 1A2, 2B6, 2C19, 3A4, 3A5, 3A7 in pmol min^{-1} pmol^{-1} of CYP; Vmax A-F = CYP A-F activity x 60 min x 30.0 mg of microsomal protein g^{-1} of liver x 27 g liver kg^{-1} of bwt, C = concentration of OP in venous blood in the liver.

According to Barter et al. (2007), Lipscomb et al. (1998) and Wilson et al. (2003) the microsomal content of human liver ranges from 21-33 mg g^{-1} liver with reports as high as 53 mg g^{-1} liver. The average adult liver has been reported to be 25.7 g kg^{-1} of bwt (Brown et al. 1997; Davies and Morris 1993).

Oral bioavailability in vivo is dependent on both permeability and first pass metabolism that may occur in the intestine and liver. The intrinsic clearance per pmol CYP is assumed to be the same in human liver microsomes (HLM) as in intestinal microsomes (Yang, J et al. 2004), whereas CYP3A abundance represents the total amount of enzyme, 70,000 pmol in the gut (Paine et al. 1997). The abundance of CYP3A in the gut is calculated using Eq. [32].

$$\textit{Individual}\ \text{CYP3A gut abundance} = \text{Average CYP3A gut abundance} * \frac{\textit{Individual}\ \text{GSA}}{\textit{Average}\ \text{GSA}} \quad [32]$$

where GSA is the surface area of the small intestine (ileum, jejunum and duodenum). The surface areas of each segment of gut are calculated from their length and diameter. To be useful in predicting absorption, the $P_{eff\ values}$ in Table 13 and the CYP3A activities (V_{max} and K_m, etc.) in the intestinal tract require use of an ACAT or CAT submodel to augment the PBPK/PD model (Simulations-Plus, Inc.).

7.5 Comparison of Predicted vs. Experimental V_{max} and K_m Values

The predicted V_{max} and K_m values for deltamethrin were compared to experimental values published by Godin et al. (2007) in Table 12 (Section 6.4), where 2C19 was the major human CYP involved. K_m was expressed in μM and V_{max} in pmol min^{-1} $pmol^{-1}$ of CYP. The experimental V_{max} value for 2C19 is equivalent to 56.3 μmol h^{-1} kg^{-1} of bwt (19 pmol mg^{-1} protein; Godin et al. 2007), while the predicted V_{max} is 0.051 μmol h^{-1} kg^{-1} of bwt (ADMET Predictor: Table 15, (1.04E-3 x 60 x 30 x 27)/1000). ADMET Predictor gave a similar human K_m value (5.20 μM) relative to the experimental value shown in Table 12 (9 μM). According to ADMET Predictor (Table 15), deltamethrin is not a CYP 2C19 substrate, but rather is a substrate of CYP3A4 (Table 18), which has a V_{max} of 6.56 μmol h^{-1} kg^{-1} of bwt, and a K_m of 41.50 μM. The P450 V_{max} value for rat liver microsomes reported by Mirfazaelian et al. (2006) was 76.1 μmol h^{-1} kg^{-1} of bwt and 74.0 μM for K_m.

In calculating V_{max}, the following in vitro to in vivo extrapolations were used: 30 mg of microsomal protein g^{-1} of liver, 27 g of liver kg^{-1} of bwt. These values may be changed and V_{max} recalculated as desired. In this calculation, the 2C19 CYP content in the microsomes was corrected by ADMET Predictor (Inoue et al. 2006).

7.6 Prediction of Liver and Plasma Carboxylesterase Activity

Two studies that addressed the use of in silico models for predicting human carboxylesterase (hCES1 and hCES2) activity have been published (Yoon et al. 2003; Vistoli et al. 2010). Yoon et al. examined the hydrolysis of the prodrug CPT-11 ([1,4'-bipiperidine]-1'-carboxylic acid, (4*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-9-yl ester; CAS no. 1255644-71-3) by carboxylesterases (CE) and CPT-11 analogs to the active metabolite SN-38 (1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione, 4,11-diethyl-4,9-dihydroxy-, (4*S*)-; CAS no. 86639-52-3), a topoisomerase I inhibitor. Comparative molecular field analysis and molecular similarity index analysis, along with docking studies were used to predict the biological activity of a derivative of CPT-11, i.e., BP-CPT (1-piperazinecarboxylic acid, 4-(phenylmethyl)-, (4*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-9-yl ester; CAS no. 97682-40-1) in U373MG glioma cell lines (Yoon et al. 2003). Docking models showed a similar orientation of the SN-38 moiety for both CPT-11 and BP-CPT in the active site of rCE. The rate of hydrolysis of CPT-11 or BP-CPT by CEs to SN-38 appears to be determined by either the proximity or the angle of the carbonyl group of the

prodrug to the hydroxyl of the Ser-221. QSAR (3D structures) studies performed on CPT-11 analogs (structures vs. relative activity of rat serum, Tsuji et al. 1991) were evaluated by CoMFA and CoMSIA analyses. Comparative molecular field analysis (CoMFA) is a mathematical expression of the correlation between the chemical structure of a series of compounds and the experimentally determined biological activities of the compounds (Cramer et al. 1988). Comparative molecular similarity index analysis (CoMSIA) is an alternative molecular field analysis method to CoMFA.

Vistoli et al. (2010) studied the docking analysis of known substrates to develop both predictive models and molecular dynamics (MD) simulations to identify the in situ behavior of substrates and products. Pyrethroids and their respective carboxylesterase K_m data (Ross et al. 2006) were used as substrates in the training set and in the external test sets for a series of docking experiments. A rich interaction pattern with hCES1 was shared by all the pyrethroid insecticides examined, and explains the marked efficacy with which the esterases hydrolyze the pyrethroids. Molecular dynamics simulations were used to examine the hydrolysis of heroin (CAS no. 561-27-3) to 6-O-acetylmorphine and morphine (CAS no. 57-27-2) by hCES1

Chang et al. (2009) reported the viability of using a consensus 3D QSAR pharmacophore model to predict the stereospecific rat serum carboxylesterase (CEs) hydrolysis rates and half lives for the pyrethroids. Utilizing a stereoselective *in vitro* metabolism dataset from Sprague-Dawley rat serum CES, and 27 Type I and II pyrethroids, a ligand-derived pharmacophore model was developed to filter catalytically "competent" and "incompetent" ligands, that were based on four pharmacophore features (i.e., two

aromatic, and two projected hydrogen bond acceptors). A concurrent pharmacophore model was further enhanced to determine the features that were common within the "incompetent" set of ligands, and these features were used to further discriminate for false positives. By filtering catalytically "competent" ligands (i.e., on the basis of hydrolysis rates > 6 h^{-1}; "fast" hydrolysis), two subsequent QSAR models were developed that demonstrated two distinct chemical descriptor spaces for the two filtered ligand sets. Docking simulations that were based on a homology-modeled rat serum carboxylesterase sequence were also performed. Further analysis via protein ligand interaction fingerprints (PLIF) showed that catalytically "competent" ligands were dominated by SER (serine) and GLY (glycine) interactions, which were consistent with the deduced projected hydrogen bond acceptor pharmacophore features in the model. Several QSAR models developed for predicting CES-mediated hydrolysis rates were suggestive of these criteria (i.e., pharmacophore features), with an emphasis on terms that were consistent with enzymatic hydrolysis. The best substrate-predicted rates gave higher significance to the carbonyl carbon atom charge, lipophilicity, and molecular dipole moment that were consistent with an enzymatic metabolism mechanism. The 31 selected stereoisomers, along with their catalytic hydrolysis rates (k_{cat}), are presented in Table 19.

[Insert Table 19 here]

The 3D QSAR pharmacophore model selected "very fast" and "very slow" metabolizers leaving the catalytic hydrolysis rates in the middle group as being questionable. Catalytic rates (k_{cat}) for the pyrethroids that were outside of the training set (8 to 25 h^{-1}) were

obtained by extrapolation. Less confidence should be placed on these predicted values. The hydrolysis rate for deltamethrin (1R, cis, αS) fell into the middle group and was not included in Table 19. However, catalytic rates (rat serum carboxylesterases) for two trans deltamethrin (1R,3S, αR; 1S,3R,αR) isomers were included in the table and compared favorably with the rat hydrolase A and B k_{cat} values in Table 11. Cypermethrin (1R, trans, αR), cyfluthrin (1R, trans, αR; 1R, trans, αR) and permethrin (1R, trans; 1S, trans) were fast metabolizers. Among the slow metabolizers were permethrin (1S, cis), cyfluthrin (1S, cis, αS) and cypermethrin (1R, trans, αS).

7.7 Conjugation of Intact Pyrethroids and Aromatic Leaving Groups

Sorich et al. (2006) evaluated the ability of glucuronosyltransferases (UGT) to transfer glucuronic acid to various substrates (drugs with hydroxy groups). ADMET Predictor contains a QSAR model developed by Kurt Enslein which predicts "Yes" or "No" answers for the glucuronidation of various substrates, based on the data provided by Sorich et al. (2006). UGT does not appear to glucuronidate the intact hydroxylated pyrethroids. Enslein's QSAR model for predicting which drugs may become glucuronidated was used to predict whether the hydroxylated pyrethroids are glucuronidated. Generally, the model responded with a "No" answer.

7.8 Skin Permeation Constants

A QSAR model was developed by Potts and Guy (1992) to predict the permeability of a wide range of structurally different chemicals, and was based upon the size of the permeant and its octanol/water partition coefficient. Flynn (1990) compiled data on ~90 compounds for which Eq. [33], below, was used to derive K_p values. The compounds ranged in molecular weight from 18 to >750, and in log K_{oct} from -3 to +6.
The results obtained by applying Eq. [33] (below) indicate that an increase in K_p with increasing K_{oct} is offset by increasing molecular size.

$$\log K_p(cm*\sec^{-1}) = -6.3 + 0.71*\log K_{oct} - 0.0061*MW \quad [33]$$

Table 20 provides the computed log K_p and K_p values for the 15 pyrethroid insecticides we address in this review, by applying Eq. [33], and by using the algorithm of Potts and Guy (1995) within QikProp. The K_p values for permethrin and phenothrin (1.967 and 3.724) were at least 10 fold larger than those of the other pyrethroids. The log K_{oct} values (7.64 and 7.68) appear to be responsible for these large values.

[Insert Table 20 about here.]

Reifenrath et al. (2011) reported that 2.1% of a 200 µg cm^{-2} dose of permethrin was absorbed through excised human skin during a 24-h period, in which 0.1% was found in the receptor fluid and 2.0% in the dermis. A dose of 2.25 µg cm^{-2} resulted in 1.3% percutaneous absorption of permethrin 24 h after 0.23% was found in the receptor fluid and 1.1% in dermis. For the 200 µg cm^{-2} dose of 0.1% in the receptor fluid, the flux was

calculated to be 200 μg cm^{-2} x 0.10 divided by 24 h = 0.833 μg cm^{-2} h^{-1}. The K_p was calculated by dividing the flux by the dose in μg cm^{-3} to give 4.164 x 10^{-3} cm h^{-1}. The K_p value (1.967 and 0.3597) for permethrin (Table 20) is closer to the flux value (0.833) calculated in the Reifenrath et al. (2011) study, whereas the K_p value (0.004164) from Reifenrath et al. (2011) approaches the flux value (0.0047) from QikProp 3.0 (Schrodinger, LLC). The reason for this appears to result from the $\log_{oct}$ values in Eq. [33] and the K_p and solubility estimates used in QikProp.

Bast et al. (1997) calculated a K_p of 2.63 x 10^{-8} cm h^{-1} for permethrin in a single pass rabbit ear perfusion study. The value (1.967) predicted by the Potts and Guy (1992) equation is 7.5 x 10^7 greater. However, the results of in vivo rat absorption studies (43 to 46% over 7 to 14 d) conducted by Sidon et al. (1988) appear to produce similar results to the high Potts and Guy (1992) K_p value for permethrin. According to Farahmand and Maibach (2009), in vitro estimates may be improved when vehicle effect is considered and studies are carried out under steady state conditions.

7.9 Tissue:Blood Partition Coefficients; QSAR Models

Knaak et al. (2008) reviewed the nonlinear QSAR equation developed by Zhang (2005) and the QSAR model developed by Liu, HX et al. (2005) for predicting tissue:blood partition coefficients. To our knowledge, neither the QSAR equations nor models are available in the form of ready-to-use programs for obtaining partition coefficients for the pyrethroids. QikProp has a program for predicting the brain/blood partition coefficients

for orally delivered drugs. The log BBB values for the 15 pyrethroids are given in Table 13.

8 Lipids, Target Proteins and Ion Channels

8.1 Biomembranes and Ion Channels

The nature of bilayer lipid membranes (biomembranes) are infrequently mentioned in papers dealing with ion channels, even though biomembranes harbor receptors, ion channels (membrane proteins), lipid domains, lipid signals, and scaffolding complexes, which function to maintain cellular growth, metabolism, and homeostasis (Sudhahar et al. 2008). In biomembranes, proteins recognize ester groups and headgroups such as choline, ethanolamine, and serine of the phospholipids and the hydroxyl and amide functions of the sphingomyelins. The 3-dimensional structures of these biomembranes are currently being examined (Kuo 1985; White 1994).

Membrane phospholipids (i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS)) play an active role in signal transduction and functional modulation. Proteins embedded in outer cell membranes (ATP-binding cassette ABC transporters; P-glycoprotein, multidrug resistance-associated protein 1 and breast cancer resistance protein) regulate the movement of chloride ions, nutrients and structurally diverse compounds across cell membranes (Robey et al. 2010). Knaak et al. (1997) reviewed the affects of drinking water and dietary levels of mono-, di-, and tri-ethanolamine in animals. A single large dose of either ethanolamine or diethanolamine

produces an increase in the formation of liver phospholipids (choline and noncholine). An LD_{50} dose (2.3 $g \cdot kg^{-1}$) of diethanolamine in the mouse produced abnormal electron microscopic changes in hepatocyte-mitochondria and in smooth and rough endoplasmic reticulum (ER). Widespread changes in mitochondria and ER suggest that cell membranes may have been damaged. The fatty acid (i.e., oleic, linoleic, linolenic and arachindonic acid) composition of phospholipids may also be affected by dietary levels of ethanolamine. The specificity of CDP-ethanolamine: 1, 2-diacyl-glycerol ethanolamine phosphotransferase was less sensitive to the chain length of the fatty acid in diacyl-glycerol than it was to their degree of unsaturation.

The following schematic (Fig. 13) depicts the structure of the lipids as they appear in biomembranes (Nikolelis et al. 1991)

[Insert Figure 13 about here.]

The relationship between biomembranes, ion channels and peripheral proteins needs to be addressed in studies involving the neurotoxicity of pyrethroids. The multiple target systems for pyrethroids that is presented in Table 21 indicate that these lipid soluble insecticides are well distributed in biomembranes and bind to the proteins (i.e., ion channels) associated with them. The extent of this distribution in not well known; however, only 1% of the pyrethroid concentration in nervous tissue is believed to be involved in the modification of ion channel proteins, with the remainder distributed in biomembranes phospholipids or bound to associated proteins.

According to O'Reilly et al. (2006), the sodium-channel binding cavity is accessible to the lipid bilayer and therefore to lipid-soluble insecticides. The properties of ion channels are measured by recording transmembrane currents using the patch-clamp technique (Horber et al. 1995).

8.2 Patch Clamp Technique

The patch clamp technique is an extension of the voltage clamp methodologies. The scope of the technique may be described by Ohm's law:

$$V = IR \quad [34]$$

where the experimenter controls the voltage (V), records current (I), and the resistance (R) is a variable dependent upon the characteristics of the cell. This is carried out by the use of a single pipette, allowing the application of this technique to cells smaller than the squid giant axon. The patch in patch clamp refers to the patch of cell membrane contained within the polished aperture (~1 μm diameter) of a glass pipette that is placed against the membrane of a single cell. Suction is applied to the pipette to form a high giga-ohm resistance seal. The amplifier controls– "clamps" – the voltage across the cell and measures the current flowing through the ion channels in the cell membrane. Single-type ion channels may be studied using solutions of specific ionic composition. The replacement of K^+ in buffers with Cs^+ allows for Na^+ channels to be studied in the

absence of contaminating K^+ current. IC_{50}s, K_{iS} and K_{DS} (inhibition and dissociation constants) may be measured for voltage-gated channels.

8.3 Voltage Dependence and Kinetics of Activation and Inactivation

The symptoms of pyrethroid poisoning are represented by hyperactivity, and are induced primarily by modulation of the sodium channels. Type I and II (without and with cyano groups) pyrethroids cause a multiple action on the gating kinetics (opening and closing of channels by proteins) of the sodium channel. The action is as follows:

1) Activation (opening of channel proteins): voltage is shifted in the hyperpolarizing (change to a more negative potential) direction.

2) Deactivation (closing): voltage relationship is shifted in the hypopolarizing direction (change to a more positive potential).

The pyrethroids cause slow activation (opening) and deactivation (closing) of the sodium channel. Type II pyrethroids increase discharge frequency in sensory neurons by membrane depolarization (change in potential to a more positive value), causing paraesthesia as observed in the arms and hands of workers dermally exposed to these insecticides.

8.4 Atomic Force Microscopy

The atomic force microscope (AFM) is currently used to examine biomembranes and membrane proteins in electrophysiological studies involving the patch-clamp technique (Danker et al. 1997). The structural information of the AFM image may be combined with the functional electrical data (Goksu et al. 2009). Danker et al. (1997) observed three kinds of membrane types, which had different surface morphologies or conducting properties such as the presence of undesired ER.

8.5 Effective Concentrations for Altering Sodium Channels

Ray and Fry (2006) recently published the content of the information presented in Table 22, which is reproduced with permission herein to show that multiple target systems exist for the pyrethroid insecticides. Effective concentrations have ranged from 10^{-4} M down to 10^{-13} M. This is below the concentrations (10 to 100 μM) used by Choi and Soderlund (2006) to study the time course of Na_v 1.8 sodium channel modification by cismethrin, and those used to study the in vitro metabolism of the pyrethroids by liver microsomes (up to 100 μM). K_m values for both oxidative and hydrolytic activities exceeded 1 μM, and were generally in the range of 20 to 75 μM (Godin et al. 2006)

[Insert Table 21 about here]

The substrate concentrations (K_{ms} for pyrethroids) agree with the concentrations of other substrates reported in the literature that have low water solubilities. The practice of using solvents to add pyrethroids to incubation media provides a means for saturating lipids in

the microsomes, and making these substrates available to enzymes associated with the lipids. Information as to whether the pyrethroids are free or bound to tissue proteins is required for use in the PBPK/PD models for determining partition coefficients, or metabolic rates. Binding also influences the results of toxicity studies, because pyrethroid insecticides bind to voltage-sensitive sodium channels and modify their gating kinetics, thereby disrupting nerve function and producing acute neurotoxic effects in both insects and nontarget organisms (Soderlund et al. 2002).

8.6 Sodium Channel Modifications

Fig. 14, adapted from Choi and Soderlund (2006), shows that sodium channel modification occurs as a function of pyrethroid concentration.

[Insert Figure 14 about here.]

Such concentrations are in the range of 1 to 100 μM, as used by Godin et al. (2006) in metabolism studies. The modification was limited at concentrations representing the solubilities (0.1 μM) in water of these two pyrethroids. The concentrations of these pyrethroids that actually exists near the active sites of the enzymes (CYPs and carboxylesterases) and sodium channels are not known. According to Choi and Soderlund (2006), determining the relative potency of pyrethroids in the assays was complicated by the low aqueous solubility of these compounds, and the complexity of the kinetics by which they partition into the large lipid-rich compartments of the oocyte membrane and

yolk. Song and Narahashi (1996) reported a toxicologically meaningful disruption of nerve function that probably occurs at concentrations causing modification of a very small proportion (<1%) of channels.

The percentage of channels modified by the pyrethroids may be calculated using Eq. [35] (Tatebayashi and Narahashi 1994):

$$M = \{[I_{tail}/(E_h - E_{Na})]/[I_{Na}/(E_t - E_{Na})]\} * 100 \qquad [35]$$

where:

I_{tail} is the maximal tail current amplitude, E_h is the potential to which the membrane is repolarized, E_{Na} is the reversal potential for sodium current determined from the current-voltage curve, I_{Na} is the amplitude of the peak current during depolarization before pyrethroid exposure, and E_t is the potential of step depolarization.

The concentration-response data may be fitted to the Hill equation (Tan et al. 2005):

$$M = \frac{M_{\max}}{(1 + (K_d/[P])^{n_H}} \qquad [36]$$

where [P] represents the concentration of pyrethroid and K_d represents the concentration of pyrethroid that produced the half-maximal effect, n_H represents the Hill coefficient, and the M_{max} is the maximal percentage of sodium channels modified.

8.7 Mitochondrial Complex in Neurotoxicity

Gassner et al. (1997) studied the effects of permethrin and cyhalothrin on the mitochondrial complex I. Our Fig. 15 was reproduced from this article (with permission) to show that the dose-response curves for permethrin and cyhalothrin on succinate-sustained O_2-consumption of rat liver mitochondria, during state 3 respiration. Half maximum inhibition (50%) occurred at 7.6 μM with permethrin, while 2.4 μM cyhalothrin resulted in 54% inhibition. The concentration is less than the K_m values for metabolism.

[Insert Figure 15 about here.]

The most sensitive response (inhibition) appeared to be in the reduction of NADH:duroquinone oxidoreductases activity, wherein half maximal inhibition occurred with 0.73 μM permethrin and 0.57 μM cyhalothrin. These results are shown in Fig. 16. The lipid content of mitochondrial protein was not stated. Duroquinone (CAS no. 527-18-4) is 2,3,5,6-tetramethyl-1,4-benzoquiunone (molar solubility = 4.3×10^{-3} mol·L^{-1} or 4300 μM).

[Insert Figure 16 about here.]

Mathematical analysis of the inhibition kinetics that occurred with permethrin and cyhalothrin indicated substantial cooperativity. Fitting the data to the equation listed

below (Eq. [37]) yielded n-values of 2.4 for permethrin and 3.5 for cyhalothrin, indicating that 3 to 4 molecules of the two pyrethroids interacted with the mitochondrial complex 1.

$$Y_{[p]} = \frac{Y_{\max}[P]^n}{K' + [P]^n} \qquad [37]$$

Eq.[37] is based on the Hill equation. At least 40 subunits of complex 1 are potential targets for binding of the pyrethroids. Model systems have recently been described that use this mode of action for the induction of neurodegenerative diseases (Gassner et al. 1997).

Soderlund et al. (2002) made the following observation: "The diverse toxic actions and pharmacological effects of pyrethroids suggest that simple additivity models based on combined actions at a single target are not appropriate to assess the risks of cumulative exposure to multiple pyrethroids."

9 Neurotoxicity

The neurotoxicity of the pyrethroids was extensively reviewed by Soderlund et al. (2002), Shafer et al. (2005), Wolansky and Harrill (2008), and Breckenridge et al. (2009). The results of the recent analysis by Breckenridge et al. (2009) support the original hypothesis that pyrethroids exert their toxicological effect through Type I (T-syndrome) (tremor) and Type II (CS syndrome) (choreoathetosis (abnormal body movements)-salivation) syndromes. The CS-syndrome is associated with α-cyano pyrethroids that have slower sodium ion channel kinetics, whereas the T-syndrome is associated with the noncyano

pyrethroids that have faster sodium ion channel kinetics. In addition, there are pyrethroids with sodium ion channel properties intermediate between the two extremes (i.e., deltamethrin (α-cyano) and bifenthrin (noncyano)) that tend to show both the T and CS syndrome. Breckenridge et al. (2009) classified the pyrethroids in Table 22 on the basis of acute neurological (FOB) functional observation battery studies (Weiner et al. 2009) and sodium (Choi and Soderlund 2006), calcium- (Symington et al. 2008), and chloride- (Burr and Ray 2004) channel studies.

[Insert Table 22 about here.]

Factor and multivariate dissimilarity analyses were used to evaluate four independent data sets from the four studies (Weiner et al. 2009; Choi and Soderlund 2006; Symington et al. 2008; Burr and Ray 2004). The analyses (factor and multivariate) by Breckenridge et al. (2009) did not provide useful information for developing the pharmacodynamic portion of a PBPK/PD model. Consequently, the original papers by Weiner et al. (2009), Choi and Soderlund (2006), Symington et al. (2008), and Burr and Ray (2004) were individually examined for useful information.

9.1 Functional Observation Batteries (FOB)

Weiner et al. (2009) identified the pyrethroids by CAS no. in his FOB studies. Absolute stereochemistry was indicated for deltamethrin, S-Bioallethrin, esfenvalerate, (λ-cyhalothrin) and relative stereochemistry for bifenthrin and tefluthrin. Stereochemistry

was not divulged for β-cyfluthrin, cypermethrin, permethrin, resmethrin, fenpropathrin and the pyrethrins. Dosages, body weights and time to peak effect provide useful information for setting oral exposure levels (gavage) and comparing peak effect times with predicted tissues concentrations from PBPK/PD models.

9.2 Na^{+1} Ion Channels

Choi and Soderlund (2006) studied the action of pyrethroids on rat $Na_v1.8$ sodium channels that are expressed in *Xenopus* oocytes. The stereochemistry of single isomers used in the study were identified by stereostructure, common name and chirality (i.e., RS, *trans*, *cis*). Analytical standards (92.9-99.8% purity) of all compounds, except for cismethrin, were utilized. Before use in this study, cismethrin was purified by preparative high-resolution liquid chromatography (99.8%) by the method of Bloomquist and Soderlund (1988). Compounds were assayed in 0.3% dimethylsulfoxide (DMSO)/97% ND-96 medium (at a final concentration of 100 μM except for deltamethrin, which had a concentration of 10 μM). Data acquisition and analysis were performed using pClamp 8.2 (Axon Instruments, Burlingame, CA) and Origin 7.0 (OriginLab Corp., Northampton, MA). Of the 11 pyrethroids studied, only three compounds other than deltamethrin (cypermethrin, cyfluthrin and cyhalothrin) exhibited statistically significant useful fractional modifications of the sodium channel.

However, all of the compounds produced clearly detectable tail currents. Tail currents reflect delayed channel deactivation (closing of channel activation gate). The partitioning

of lipid soluble pyrethroids into the lipid-rich compartments of the oocyte membrane and yolk prevented assessment of what the actual concentrations were in the concentration-effect curves. In vivo, pyrethroids bind to plasma proteins making them unavailable to lipids. Determining partition coefficients that involve lipids, and binding measurements that involve active channel protein(s), and also defining tail current decay times, may be useful in building PBPK/PD models.

9.3 Ca^{+2} Ion Channels

Symington et al. (2008) used in vitro techniques to study the influence of 11 pyrethroids on Ca^{2+} influx and glutamate release by rat brain synaptosomes. The low molar concentrations of pyrethroids used in this study were similar to the brain concentrations that were predicted by Mirfazaelian et al. (2006) using a PBPK model. The authors reported the isomeric composition of the commercial pyrethroids (bifenthrin, S-bioallethrin, cismethrin, β-cyfluthrin, λ-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, permethrin and tefluthrin) used in the study. The Hill equation was used by Symington et al. (2008) to calculate the slope of the Ca^{2+} influx.

9.4 Cl^{-1} Ion Channels

The activity of 14 different pyrethroids (10 μM concentrations) on voltage-gated chloride ion channels was investigated by Burr and Ray (2004). Patch-clamp techniques were used in their study. The hardware comprised an Axon Instrument Axopatch-1D, with

CV4-1/100 Headstage and Axon Digidata 1200 data acquisition digitizing board, routed through a personal computer. The CAS no., isomeric details, and purity of each pyrethroid (bifenthrin, esbiol, bioresmethrin, b-cyfluthrin, cyfluthrin isomer 2, cyfluthrin isomer 4, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, permethrin, resmethrin and tefluthrin) was given. Open channel probability values were reported. Overall, 3/6 of the Type II pyrethroids, 2/2 of the mixed-type pyrethroids, and 0/3 of the Type I pyrethroids were active at the chloride ion channel. The type of three of the 14 pyrethroids were not disclosed. The results (probability values) were not considered to be useful in the context of PBPK/PD models.

9.5 Developmental Neurotoxicity

Shafer et al. (2005) reported that there is no information on age-dependent toxicity for most pyrethroids. Their review summarizes the results of 22 studies performed on the developmental neurotoxicity of pyrethroids (strengths and limitations of studies). Many of the studies suffer from inadequate study design, problematic statistical analysis, use of formulated products (e.g., unknown isomer mixtures), and/or inadequate controls.

Several research needs were pointed out in this study. These included the additional need for information on the potential differences underlying age-dependent sensitivity to the pyrethroids, clarification of changes in behavioral and biochemical endpoints, and linking these end points to voltage-sensitive sodium channels (VSSCs) or other cellular targets. The authors suggest that BBDR (Biologically-based Dose-response Models) be

developed (Andersen and Dennison 2001) that describe the relationship between different components of the continuum between exposure to, and the adverse effects of, a chemical. A model of this nature was constructed for the developmental neurotoxicity of perchlorate (Jarabek, 2002). A PBPK model that adequately describes the relationship between exposure and target tissue dose is also needed.

10 Discussion

The sections on the metabolism and neurotoxicity of the pyrethroids in this review provide a starting point that feeds into the physiological and biochemical parameters that are needed to develop PBPK/PD models for assessing risks to the pyrethroids. The development of such pyrethroid model parameters requires knowledge of their discovery, chemistry, chirality, their isomers and their chromatographic separation. To this end, Section 2 above (viz., Nature of Pyrethroid Insecticides) was developed with a listing of 15 of the most important pyrethroids available, their isomers, technical products and aspects of their analytical chemistry. The detailed information on these 15 pyrethroids is presented in Appendices B and C, along with their NMR spectra, which shows the double bond of the chrysanthemic acid being in the Z position. The gem-dimethyl group on the cyclopropane ring is essential, because those compounds without it are essentially inactive. A side chain on the alcoholic moiety is also necessary. Unsaturation in the side chain of the alcohol may be alkenyl, cycloalkenyl or aromatic (furyl, benzyl, or phenoxy) and is important to the biological activity of these insecticides.

The absolute stereochemistry of the active isomers (i.e., registered commercial products) is given in Appendix B. Registered products may contain all of the known isomers or only several of the more active isomers. Alpha-cypermethrin, a well-known product used on cotton in Egypt, contains a 50:50 mixture of two *cis* isomers [(1R, 3R, S) and (1S, 3S, R)], whereas deltamethrin contains only one *cis* isomer [(1R, 3R, S)] as the active ingredient. The wording on a label of a product (Bisect L, Loveland Products, Inc., Greeley, CO) containing 7.9% bifenthrin states that the product contains *cis* isomers 97% minimum, and *trans* isomers 3% maximum. The absolute configuration of these isomers (*cis* and *trans*) are given in Table B3, Appendix B. Over the last 10 years, the development of chiral columns (HPLC and GC) has made it possible to easily separate the pyrethroid isomers. The lag in the development of analytical methodology and the relative lack of availability of metabolic standards has discouraged many investigators from undertaking pyrethroid studies, in particular metabolism and electrophysiological studies, in which isomers and their metabolic products need to be identified and quantitated.

PBPK models (i.e., GastroPlus, SimulationsPlus, Inc., Lancaster, CA) that are used in the pharmaceutical industry include code for the ACAT models, which describe the passage of a drug through the GI tract (Yu et al. 1996). The CAT models require additional parameters (e.g., length, radius, transit time, and pH for GI tract, regional effective drug permeability (P_{eff}), solubility, absorption scale factors and metabolic data that are not required by simple gastrointestinal absorption models (i.e., rates of absorption from stomach and GI tract) for use in most pesticide-based PBPK/PD models. An ACAT

PBPK/PD model has not been used as yet to describe the absorption of pyrethroids by the GI tract. Pyrethroid solubility data are available, and investigators at the University of Georgia, Athens, are currently developing permeability data involving P-glycoprotein using Caco-2 cells. V_{max} and K_m values obtained from work on liver enzymes may be used to simulate metabolism by gut enzymes based on their regional content in the GI tract.

The percutaneous absorption of pyrethroids is described in terms of their permeability to skin ($K_{p,}$ cm^{-1}); such permeability constitutes their rate of movement from the surface of the skin, through the stratum corneum, to the capillary bed. Bast et al. (1997) calculated a K_p value of 2.63 x 10^{-8} $cm \cdot h^{-1}$ for permethrin. Moreover, Bast et al. (1997) commented on the high permeability data of Sidon et al. (1988) involving permethrin, which they thought defied interpretation. From the QSAR equation of Potts and Guy (1992) a percutaneous K_p ($cm\ h^{-1}$) for permethrin was predicted to be 1.967. This value is 7.5 x 10^7 greater than the value reported by Bast et al. (1997). Additional work is needed to determine whether the high results of Sidon et al. (1988) are inconsistent with the Bast el al. (1997) value. At present the absorption K_ps for the pyrethroids appear to be extremely low, based on the rat in vivo studies of Scott and Ramsay (1987) with cypermethrin and in vitro studies with isolated rat and human skin. The in vitro percutaneous studies of Reifenrath et al. (2011), performed on human skin with permethrin, support these observations.

The mechanistic models of Poulin and Theil, Berezhkovskly and Rodgers, Leahy, and Rowland provide non-laboratory approaches for predicting tissue:blood partition coefficients for nonvolatile chemicals, such as the pyrethroids and their metabolites. Although this procedure may not be as precise as the laboratory methods developed by Gargas et al. (1989) for volatile chemicals, or the procedure of Jepson et al. (1992, 1994) for nonvolatiles, the values may be easily calculated and recalculated as PBPK/PD modeling is performed, and later can be compared to values developed in the laboratory. The parameters inputted to the Poulin and Theil and Berezhkovskly partitioning models included a value for F_{up} (% unbound in plasma) for calculating f_{up} and the ratio of $f_{up}/f_{ut,}$ which was used as a multiplier for correcting the lipid partition coefficients. This correction substantially reduced the pyrethroid and pyrethroid metabolite partition coefficients determined for fat, as shown in the tables of Appendix C. The Rodgers, Leahy and Rowland equations for the binding of basic drugs with acidic phospholipids, and the binding of neutral-acidic drugs with albumin, were not used to determine the partition coefficients of the pyrethroids or their metabolites (neutrals and acids), because plasma binding was adequately handled by the Poulin and Theil and Berezhkovskly partitioning and binding models.

The generic structures, technical names, physical and chemical properties, and tissue partition coefficients of the 15 pyrethroids and their metabolites are provided in tables D1-D15 of Appendix D. Physical property values for modeling were obtained using a 2D model, ACD 12 (Advanced Chemistry Development, Inc., Toronto, Canada). A QSAR 3D model (i.e., QikProp 3.0 (Schrodinger, LL)), used in the development of F_{up} (fraction

unbound to plasma protein), indicated that the differences in physical binding properties between isomers were small. Differences, however, exist in chemical properties as noted in metabolism studies reviewed in Section 5. Biotransformation and elimination paths for the pyrethroids that are presented in tables E1-E15 of Appendix E incorporate estimated metabolic rate data for PBPK/PD model development.

The importance of chirality (*cis*, *trans* isomers, etc.) to the pyrethroids was recognized early by Abernathy and Casida (1973), Casida et al. (1971a), and Casida et al. (1975) as they conducted studies on houseflies, mice, or rats, or esterase and oxidase systems derived from the organisms. Microsomal P450 enzymes oxidize the (+)-*trans*-chrysanthemate moiety at the *trans*-methyl group of the isobutenyl (propenyl) substituent and at one of the gem-dimethyl groups (2-(hydroxymethyl)-2-methyl-cyclopropane), while the (+)-*cis*-isomer is oxidized at either of the isobutenyl methyl groups. Products isomerized at C3 of the cyclopropane moiety are detected only after ester cleavage and oxidation of an isobutenyl methyl group. The alcohols are oxidized at various sites that depend upon whether they possess pentadienyl, allyl, benzylic methylene, or aromatic substituents. The presence of chloro and trifluoro groups (2-chloro-3,3,3-trifluoro-1-propenyl) on the isobutenyl group of bifenthrin, cyhalothrin and tefluthrin result in the hydroxylation of one of the gem-dimethyls and one or more positions on their alcohols (aromatic, benzylic, etc.).

Hydrolysis of the pyrethroids may occur prior to hydroxylation. For dichloro groups (i.e., cyfluthrin, cypermethrin and permethrin) on the isobutenyl group, hydrolysis of the

trans-isomers is the major route, and is followed by hydroxylation of one of the gem-dimethyls, the aromatic rings and hydrolysis of the hydroxylated esters. The *cis*-isomers are not as readily hydrolyzed as the *trans*-isomers and are metabolized mainly by hydroxylation. Metabolism of the dibromo derivative of cypermethrin, deltamethrin, is similar to other pyrethroids (i.e., cyfluthrin, cypermethrin and permethrin) that possess the dichloro group. Type II pyrethroid compounds containing cyano groups (i.e., cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin and fluvalinate) yield cyanohydrins (benzeneacetonitrile, α-hydroxy-3-phenoxy-) upon hydrolysis, which decompose to an aldehyde, SCN ion and 2-iminothiazolidine-4-carboxylic acid (ITCA). Chrysanthemic acid or derivatives were not used in the synthesis of fenvalerate and fluvalinate. The acids (i.e., benzeneacetic acid, 4-chloro-α-(1-methylethyl) and DL-valine, *N*-[2-chloro-4-(trifluoromethyl) phenyl]-) were liberated from their esters and further oxidized/conjugated prior to elimination. Fenpropathrin is the only pyrethroid that contains 2,2,3,3-tetramethyl cyclopropane-carboxylic acid. The gem-dimethyl is hydroxylated prior to or after hydrolysis of the ester and is oxidized further to a carboxylic acid prior to elimination.

Phenothrin, resmethrin and tetramethrin are Type I compounds possessing 2,2-dimethyl-3-(2-methyl-1-propen-1-yl)-cyclopropanecarboxylic acid. The gem-dimethyls remain largely untouched except for *cis*-phenothrin, while the (2-methyl-1-propen-1-yl) groups are oxidized to alcohols and ultimately to carboxylic acids. The alcohol moieties are hydroxylated or sulfonated prior to ester hydrolysis. The metabolites of the pyrethroids (i.e., acids and alcohols) are largely conjugated with glucuronic or sulfuric acids prior to

being eliminated in urine. No evidence was obtained to indicate that intact hydroxylated esters were conjugated.

PBPK/PD models require metabolic rate constants (V_{max} and K_m) adequate to describe the hydrolytic and oxidative (hydroxylation) metabolism of the pyrethroids in humans, rats and other mammals that are used in modeling. Appendices D and E provide physical/chemical descriptions of the pyrethroids, their degree of elimination, and their metabolites or degradation products along with their pathways (i.e., rate of metabolism, RAM, etc.) in rat tissues. Hydrolysis by hepatic microsomal carboxylesterase (CEs) of the parent pyrethroid is generally considered to be the first and most important step in the biotransformation in mammals. Ross et al. (2006) determined the rate of metabolism of several Type I (i.e., bioresmethrin > *trans*-permethrin > *cis*-permethrin) and Type II (i.e., deltamethrin > alpha-cypermethrin) pyrethroids by using rabbit rCEs to measure rates of product formation (acid and alcohol leaving groups). Metabolic rate constants for these pyrethroids were also examined for human hCE-1, hCE-2, and two pure rat CEs (hydrolase A and B). Protein expression of hCE-1 was determined in human liver microsomes using anti-hCE-1. Ross et al. (2006) concluded that hepatic microsomes from rats, mice, and humans were similarly active in hydrolyzing the pyrethroids studied. No information was provided concerning the activity of the CEs in rat or human sera against the pyrethroids, or their action on hydroxylated pyrethroid esters. Crow et al. (2007) extended the work of Ross et al. (2006) to include the expression and hydrolytic activity of CEs present in the cytosol and various other rat and human tissues, including rat and human intestinal microsomes and serum. The hydrolysis products detected after

HPLC analysis included the parent acids and alcohols of bioresmethrin, deltamethrin and *trans*-permethrin. Human serum was found to lack pyrethroid hydrolytic activity. Variable amounts of hCE-1 protein were observed to exist in cytosolic samples, in contrast to the nonvariable levels found in human microsomes. Chang et al. (2009) developed k_{cat} values for the hydrolysis of pyrethroids by rat serum carboxylesterase, using a consensus 3D QSAR pharmacophore model. These values have not yet been used in any published PBPK/PD model.

Using the parent compound depletion method, pyrethroid metabolic rate constants (i.e., V_{max} and K_m, k_{cat}, etc.) for hydroxylation by cytochrome P450 enzymes or hydrolysis by carboxylesterases were developed by Scollon et al. (2009). The sources of the enzymes were rat and human microsomes. The pyrethroids they studied included bifenthrin, S-bioallethrin, bioresmethrin, β-cyfluthrin, cypermethrin, *cis*-permethrin and *trans*-permethrin. The depletion method considers multiple hydroxylations as a single biotransformation at sites on either the acid or alcohol moieties, or on a combination of both. The metabolic pathways (Tables D1-D15 and E1-E15 of Appendices D and E, respectively) require V_{max}, K_m and k_{cat} values for the individual hydroxylated and hydrolyzed products. It is interesting that only bioresmethrin and cypermethrin per se were found to actually be hydrolyzed.

Supersomes from BD Biosciences containing rat (i.e., CYP1A1, 1A2, 2A1, 2B1, 2C6, 2C11, 2C13, 2D1, 2D2, 3A1 and 3A2) or human CYPs (i.e., 1A1, 1A2, 2B6, 2CD8, 2C9*1, 2C9*2, 2C9*3 and 3A4) were individually used to obtain the percentage of each

pyrethroid (0.5 μM) metabolized by 10 pmol of P450, during a 10-min incubation period. The results are interesting, but to be useful in PBPK/PD models, rate constants (pmol min^{-1} $pmol^{-1}$ of CYP), CYP content (pmol mg^{-1} of microsomal protein) and mg of microsomal protein g^{-1} of liver are needed to make in vitro to in vivo extrapolations.

According to Anand et al. (2006), CYP450-catalyzed metabolism of deltamethrin in the rat exceeds that by either plasma or liver carboxylesterases. The velocity of the reaction was expressed in nmol deltamethrin disappearance h^{-1} g^{-1} liver or plasma. This information was used to construct a PBPK/PD model in the adult male rat (Mirfazaelian et al. 2006). Godin et al. (2006) examined species differences between rat and human liver microsomal carboxylesterases. A significant species difference was noted in the in vitro biotransformation of deltamethrin, due in part to differences in the rate of hydrolysis by human liver microsomes. Godin et al. (2007) identified the rat and human CYP450 isoforms, and rat serum esterases that metabolize deltamethrin and esfenvalerate. Differences in the rates of hepatic oxidative metabolism were related to expression levels (abundance) of the individual P450 isoforms rather than their specific activity.

Godin et al. (2006) recognized that the rat may not be a good model for understanding the metabolism of pyrethroids in humans. Their conclusions on this point were based only on hydrolysis and oxidation of the parent pyrethroid (i.e., deltamethrin), rather than on the full metabolic pathways of the pyrethroids as outlined in Appendices D and E and in the context of a PBPK/PD modeling. We do agree, however, that a rat PBPK/PD model per se may not be suitable for extrapolating the results to humans, because of differences in

biochemical (i.e., enzymes, their activities, specific content, etc.) and physiological parameters between the species. There is still disagreement as to the microsomal content of liver (mg g^{-1} of liver) and the content of the individual CYPs per mg of microsomal protein in humans (Barter et al. 2007; Brown et al. 1997; Davies and Morris 1993; Houston 1994; Iwatsubo et al. 1997; Lipscomb et al. 1998; Wilson et al. 2003).

The tables in Appendix E were developed to provide modelers with complete information on the rat metabolic pathway for each of the pyrethroid insecticides. The tables in Appendix D are designed to provide the tissue:blood partition coefficients for the parent pyrethroids and their metabolites. Some metabolic rates may not exist for either rat or human metabolic pathways, and hence, modelers may have to zero out this parameter in the PBPK/PD models they work with. In any case, the importance of each metabolic pathway needs to be determined from the enzymes that exist in tissues, their rate constants (i.e., V_{max} and K_m), and the amount or content of each enzyme present. If in vitro enzyme work is to be performed, good analytical methods and analytical samples for each metabolite are essential.

Despite the fact that over 6,000 biological equations have been developed, there are still no QSAR models that relate chemical structure to neurotoxicity (Hansch et al. 1995). Models that relate structure to effects (i.e., Enslein 1998; Klopman and Rosenkranz 1995; Porcelli et al. 2007) have focused on broad-based toxic end points such as carcinogenesis, mutagenesis, irritation, teratogenesis, and cholinesterase inhibition. Researchers and others need neurotoxicity models that address ion channels, or another end point closely

related to the toxicity of the pyrethroids, such as the results of functional observation batteries (FOB). QSAR models that provide LD_{50} values, such as TOPKAT, are useful to modelers for selecting dosages to use in PBPK/PD models. QuikProp (3D model) and the kinetic models for recombinant human cytochrome P450 enzymes 1A2, 2C9, 2C19, 2D6, and 3A4 in SimulationsPlus Inc.'s "ADMET Predictor" appear to be the only models commercially available for predicting MDCK, Caco-2, log BBB or the individual CYP V_{max} and K_m values for use in PBPK/PD models. To our knowledge, the previously mentioned parameters are not being used in pyrethroid PBPK/PD models. The addition of rCYP V_{max} and K_m data from recent studies performed on the pyrethroids (Enslein 2010) may offer improvements over the predicted values obtained using ADMET Predictor 5.5.

Literature K_m values, derived from liver microsomal studies, are rarely adjusted for the fraction of unbound pyrethroid remaining in the microsomes. According to Youdim et al. (2008), the use of equilibrium dialysis to generate accurate protein-binding measurements (fu_{mic}, unbound drug in microsomes) is especially important for highly bound drugs. F_{up} (unbound pyrethroids in plasma) value measurements reported in this review that are used to calculate partition coefficients provide evidence that the pyrethroids are highly bound to plasma proteins. The F_{up} and Fu_{mic} values published by Youdim et al. (2008) provided data for 11 drugs that show Fu_{mic} values to be larger (unbound drug in microsomes > than unbound drug in plasma) than F_{up} values. By definition, F_{up} values represent only protein binding and not a combination of protein binding and lipid partitioning (f_{up}). Youdim and Dodia (2010) used the product-formation approach to determine V_{max} and K_m values; they then multiplied the K_m by the Fu_{mic} values to obtain

the unbound K_m-value. If we multiply the F_{up} value (0.02422) for bifenthrin by its K_m value (~5.42 μM), the new K_m is 0.131 and V_{max}/K_m ratio increases from 0.118 to 4.885. The Fu_{mic} value for bifenthrin is probably larger than the F_{up} value. However, the example using the calculated F_{up} value certainly supports the work of Youdim et al. (2008) and Youdim and Dodia (2010) for obtaining values for Fu_{mic} and using them to correct the K_m.

To our knowledge equilibrium dialysis has not been used to measure unbound bifenthrin or that of any other pyrethroids studied, in microsomes. Wang et al. (2010) reported that the FDA (Food and Drug Administeration) considers stereoisomers to be distinct chemical entities, if they are biologically distinguishable. For example, racemic amlodipine (CAS no. 88150-42-9) is active in the treatment of hypertension, but also causes peripheral edema, while S-amlodipine (CAS no. 103129-82-4), administered at half the dose, is as effective as the racemic mixture, but produces negligible edema. Similar examples of activity, inactivity, and toxicity linked to target or off target-effects probably also occur with the pyrethroids. However, the inability to separate, identify and carefully study the individual isomers has prevented researchers from definitively studying their toxicity, especially to undertake work on their inhibition or activation of ion-channel gating proteins.

The development of human CYP based QSAR models by Enslein (ADMET Predictor 5,5, SimulationsPlus, Inc.) for predicting drug-hydroxylation rate constants (i.e., V_{max} and K_m) now provides pharmacologists and toxicologists with QSAR models for obtaining rate constants while working on PBPK/PD models. We believe this is an important step

in securing a place for QSAR in toxicology and PBPK/PD model development. It is probable that these predictive QSAR models will be updated as new V_{max} and K_m data become available. Plans are currently being made to add new rate constants from the kinetic studies mentioned in this review.

Studies on the in vivo distribution and fate of pyrethroids in membranes are needed to better understand how these insecticides partition into lipids, bind to channel proteins and ultimately produce neurotoxicity by affecting how ions move through nerve membranes. Since 1962, many voltage-gated channel studies that have utilized intracellular microelectrodes and patch-clamping techniques have been performed. Patch-clamping has proved to be an essential tool for studying the electrochemical properties of membranes and their protein components. New technologies, such as nanofabricated scanning electrochemical microscopy and atomic force microscopy (SECM-AFM), may be capable of recording signals across individual ion channels while providing real-time structural information (Goksu et al. 2009).

Currently, combining AFM with the nuclear patch clamp is useful in disclosing the structural correlates of nuclear patch clamp currents. The finding that different nuclear envelope membrane configurations may occur at the tip of the patch pipette could help to explain conflicting results. To our knowledge, patch-clamped membranes exposed to the pyrethroids have not been examined by AFM to obtain structural data, in conjunction with functional electrical data. Waxman et al. (2004) reported at least nine different genes that encode distinct voltage-gated Na^+ channels (Na_v 1.1-Na_v 1.9), and all share a common structure, although different amino acid sequences, voltage-dependencies and

kinetics exist for them. Na_v 1.1, Na_v 1.2, Na_v 1.3 and Na_v 1.6 channels are found in the nervous system, Na_v 1.7, Na_v 1.8 and Na_v 1.9 channels are expressed within the dorsal root ganglion and trigeminal ganglion neurons. Na_v 1.4 and Na_v 1.5 channels are found within somatic and cardiac muscle, respectively. In most unmyelinated axons, ion channels are distributed uniformly along the axon to facilitate stable propagation of action potentials. Several models (i.e., stochastic Hodgkin-Huxley and compartments models) are used to simulate nerve impulse (action potential) propagation (Zeng and Tang 2009).

Na^+ channel dysfunctions present themselves under various clinical conditions (i.e., ischemia, drug use, electrolyte imbalance) and may cause life-threatening arrhythmias (Tan et al. 2003). Some aspects of cardiovascular research may be useful in exploring the combined or separate effects of drug and/or pyrethroid-based pharmacological Na^+ channel blockage. So far pyrethroid neurotoxicity is based largely on acute neurotoxicity and on ion channel studies, with few if any whole animal or organ pharmacological studies having been performed. Tatebayashi and Narahashi (1994) and Tan et al. (2005) suggested using the maximal tail current amplitude as a means of estimating the percentage of channels modified by the pyrethroids.

11 Recommendations

On the basis of this pyrethroid parameter review a number of data gaps became obvious. We recommend the following work be performed to improve the scientific value of QSAR and PBPK/PD models:

1) Synthesize analytical standards for hydroxylated pyrethroids (enantiomer pairs) and develop chromatographic methods for separating and identifying them.

2) Develop in vitro metabolic rate constants (V_{max} and K_m) for the CYP-catalyzed hydroxylation of parent pyrethroids (i.e., active pyrethroid isomers, enantiomer pairs), and the carboxylesterase-catalyzed hydrolysis of parent and hydroxylated pyrethroids. Species-specific metabolic and stereoisomer-specific constants for pyrethroids, particularly for human models are needed to support risk assessment efforts.

3) In vitro metabolic rate constants (i.e., V_{max}, nmol min^{-1} $nmol^{-1}$ P450) should be converted to in vivo values (μmol h^{-1} kg^{-1} bwt, Inoue et al. 2006) and be examined for their validity in model runs.

4) Plasma protein binding should be accounted for in the development of tissue:blood partition coefficients; and microsomal and CYP protein binding in the development of metabolic rate constants. Plasma protein binding was accounted for in the development of tissue:blood partition coefficients in Appendix D (Berezhkovskly 2004a; Poulin-Thiele 2000). K_m values may need to be adjusted for any nonspecific microsomal or CYP binding that occurs during kinetic measurements (Austin et al. 2002).

5) Diffusion-limited tissue parameters should be considered and developed (Mirfazalian et al. 2006).

6) The recovery of low to high percentages of oral dosages in the stomach, small and large intestine suggests that additional compartment and parameters (Caco-2-cell permeation constants, enterocytic metabolism and enterohepatic circulation) may be needed to adequately describe gut absorption.

7) In vitro ion channel electrophysiological studies are the major studies being performed now and in the past with the pyrethroids. The authors of these studies have not determined the distribution/concentration or fate of the pyrethroids (single isomers) in biomembranes that surround the ion channels being evaluated. Estimates indicate that only 1% of the applied pyrethroids are bound to proteins in the ion channels, with the remaining pyrethroids distributed or bound to other proteins. The structure of the "patch" used in pClamp studies needs to be reexamined using Atomic Force Microscopy to ensure biological consistency across ion channel preparations. Tail current measurements have been suggested as a measure of the % of modified channels. These measurements, although suggested by a number of investigators, have not been used in PBPK/PD models to describe the pharmacodynamics of pyrethroids in nerve tissue, although they should be. The toxicological relationship between the results of the functional observation batteries (FOB) and the in vitro electrophysiological studies needs to be further examined.

12 Summary

In this review we have examined the status of parameters required by pyrethroid QSAR-PBPK/PD models for assessing health risks. In lieu of the chemical, biological,

biochemical and toxicological information developed on the pyrethroids since 1968, the finding of suitable parameters for QSAR and PBPK/PD model development was a monumental task. The most useful information obtained came from rat toxicokinetic studies (i.e., absorption, distribution and excretion), metabolism studies with ^{14}C-cyclopropane- and alcohol- labeled pyrethroids, the use of known chiral isomers in the metabolism studies and their relation to commercial products. In this review we identify the individual chiral isomers that have been used in published studies and the chiral HPLC columns available for separating them. Chiral HPLC columns are necessary for isomer identification, and for developing kinetic values (V_{max} and $K_{m)}$ for pyrethroid hydroxylation. Early investigators synthesized analytical standards for key pyrethroid metabolites, and these were used to confirm the identity of urinary metabolites, by using TLC. These analytical standards no longer exist, and must be resynthesized if further studies on the kinetics of the metabolism of pyrethroids are to be undertaken.

In an attempt to circumvent the availability of analytical standards, several CYP450 studies were carried out using the substrate depletion method. This approach does not provide information on the products formed downstream, and may be of limited use in developing human environmental exposure PBPK/PD models that require extensive urinary metabolite data. Hydrolytic standards (i.e., alcohols and acids) were available to investigators who studied the carboxylesterase-catalyzed hydrolysis of several pyrethroid insecticides. The data generated in these studies are suitable for use in developing human exposure PBPK/PD models.

Tissue:blood partition coefficients were developed for the parent pyrethroids and their metabolites, by using a published mechanistic model introduced by Poulin and Thiele (2002a,b) and log $D_{pH7.4}$ values. The estimated coefficients, especially those of adipose tissue, were too high and had to be corrected by using a procedure in which the proportion of parent or metabolite residues that are unbound to plasma albumin is considered, as described in the GastroPlus model (SimulationsPlus, Inc., Lancaster, CA). The literature suggested that K_m values be adjusted by multiplying K_m by the substrate (decimal amount) that is unbound to microsomal or CYP protein. Mirfazaelian et al. (2006) used flow- and diffusion-limited compartments in their deltamethrin model. The addition of permeability areas (PA) having diffusion limits, such as the fat and slowly perfused compartments, enabled the investigators to bring model predictions in line with in vivo data.

There appears to be large differences in the manner and rate of absorption of the pyrethroids from the gastrointestinal tract, implying that GI advanced compartmental transit models (ACAT) need to be included in PBPK models. This is especially true of the absorption of an oral dose of tefluthrin in male rats, in which 3.0 to 6.9%, 41.3 to 46.3% and 5.2 to15.5% of the dose is eliminated in urine, feces and bile, respectively (0-48 h after administration). Several percutaneous studies with the pyrethroids strongly support the belief that these insecticides are not readily absorbed, but remain on the surface of the skin until they are washed off. In one particular study (Sidon et al. 1988) it the high levels of permethrin absorption through the forehead skin (24-28%) of the monkey was reported over a 7 to 14-d period. Wester et al. (1994) reported an absorption

of 1.9% of pyrethrin that had been applied to the forearm of human volunteers over a 7-d period.

QSAR models capable of predicting the binding of the pyrethroids to plasma and hepatic proteins were developed by Yamazaki and Kanaoka (2004), Saiakhov et al. (2000), Colmenarejo et al. (2001), and Colmenarejo (2003). QikProp (Schrodinger, LLC) was used to obtain F_{up} values for calculating partition coefficients and for calculating permeation constants (Caco-2, MDCK and log BBB). ADMET Predictor (SimulationsPlus Inc.) provided V_{max} and K_m values for the hydroxylation of drugs/pyrethroids by human liver recombinant cytochrome P450 enzymes making the values available for possible use in PBPK/PD models. The Caco-2 permeability constants and CYP3A4 V_{max} and K_m values are needed in PBPK/PD models with GI ACAT sub models. Modeling work by Chang et al. (2009) produced rate constants (k_{cat}) for the hydrolysis of pyrethroids by rat serum carboxylesterases. The skin permeation model of Potts and Guy (1992) was used to predict K_p values for the dermal absorption of the 15 pyrethroids.

The electrophysiological studies by Narahashi (1971) and others (Breckenridge et al. 2009; Shafer et al. 2005; Soderlund et al. 2002; Wolansky and Harrill 2008) demonstrated that the mode of action of pyrethroids on nerves is to interfere with the changes in sodium and potassium ion currents. The pyrethroids, being highly lipid soluble, are bound or distributed in lipid bilayers of the nerve cell membrane and exert their action on sodium channel proteins. The rising phase of the action potential is caused by sodium influx (sodium activation), while the falling phase is caused by sodium

activation being turned off, and an increase in potassium efflux (potassium activation). The action of allethrin and other pyrethroids is caused by an inhibition or block of the normal currents. An equation by Tatebayashi and Narahashi (1994) that describes the action of pyrethroids on sodium channels was found in the literature. This equation, or some variation of it may be suitable for use in the PD portion of pyrethroid PBPK models.

Acknowledgements

Our thanks are extended to Prof. Herbert N. Nigg, University of Florida, and Prof. James R. Olson, The State University of New York at Buffalo, for reviewing the manuscript of this article and making helpful suggestions that improve its quality. We also thank SRA International, Las Vegas, NV, for their skilled work in preparing this manuscript for publication and Dr. Robert Fraczkiewic, Simulations-plus, Lancaster, California, for calculating the Predicted V_{max} and K_m values for the CYP450s using ADMET Predictor. . The work on this review was funded by the U.S. EPA through General Services Administration Contract GS-35F-43570, tasks EP0THO00393 and EP11HO00301 with General Dynamics Information Technology (GDIT), Henderson, NV. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy, nor does it represent the official views of GDIT. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

Abernathy CO, Casida JE (1973) Pyrethroid insecticides: Esterase cleavage in relation to selective toxicity. Science 179:1235-1236.

Ackermann P, Bourgeois F, Drabek J (1980) The optical isomers of alpha-cyano-3-phenoxybenzyl 3-1,2-dibromo-2,2-dichloroethyl-2,2-dimethylcyclopropanecarboxylate and their insecticidal activities. J Pestic Sci 11:169-179.

Agoram B, Woltosz WS, Bolger MB (2001) Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliver Rev 50:S41-S67.

Allen S, Leah A (1990) Lambda-cyhalothrin: acute oral toxicity study in the rat. Zeneca Agricultural Products Laboratory No. CTL/P/3209:AR4840, Wilmington, DE.

Anadon A, Martinez-Larranaga MR, Fernandez-Cruz ML, Diaz MJ, Fernadez MC, Martinez MA (1996) Toxicokinetics of deltamethrin and its 4'-OH-metabolite in the rat. Toxicol Appl Pharmacol 141:8-16.

Anand SS, Bruckner JV, Haines WT, Muralidhara S, Fisher JW, Padilla S (2006) Characterization of deltamethrin metabolism by rat plasma and liver microsomes. Toxicol Appl Pharmacol 212:156-166.

Andersen ME, Dennison JE (2001) Mode of action and tissue dosimetry in current and future risk assessments. Sci Total Environ 274(1-3):3-14.

Angerer J, Ritter A (1997) Determination of metabolites of pyrethroids in human urine using solid-phase extraction and gas chromatography-mass spectrometry. J Chromatogr 695:217-226.

Artursson P, Palm K, Luthan K. (1996) Caco-2 monolayers in experimental and theoretical predictions of drug transport. Adv Drug Deliver Rev 22:67-84.

Audegond L, Collas E, Glomot R (1981a) RU 25474 (tralomethrin) single administration study by oral route in the rat. Study No. RU-4BE-81.240/A, Roussel-Uclaf, Paris.

Audegond L, Collas E, Glomot R (1981b) RU 25474 (tralomethrin) single administration study by oral route in the rat. Study No. 81240 DS/84/A, Roussel-Uclaf, Paris.

Austin RP, Barton P, Cockroft S L, Wenlock MC, Riley RJ (2002) The influence of nonspecific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties. Drug Metab Dispos 30:1497-1503.

Baker H, Kligman AM (1967) Measurement of transepidermal water loss by electrical hygrometry. Arch Dermatol 96:441-452.

Barbero AM, Frasch HF (2009) Pig and guinea pig skin as surrogates for human in vitro penetration studies: A quantitative review. Toxicol in Vitro 23:1-13.

Barrueco D, Herrera A, Caballo C, Delapena E (1992) Cytogenic effects of permethrin in cultured human-lymphocytes. Mutagenesis 7, 433-437.

Barter ZE, Bayliss MK, Beaune PH, Boobis AR, Carlile DJ, Edwards RJ, Houston JB, Lake BG, Lipscomb JC, Pelkonen OR, Tucker GT, Rostami-Hodjegan A (2007) Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. Curr Drug Metab 8:33-45.

Bast GE, Taeschner D, Kampffmeyer HG (1997) Permethrin absorption not detected in single-pass perfused rabbit ear, and absorption with oxidationof 3-phenoxybenzyl alcohol. Arch Toxicol 71:179-186.

Baynes RE, Halling KB, Riviere JE (1997) The influence of Diethyl-m-toluamide (DEET) on the percutaneous absorption of permethrin and carbaryl. Toxicol Appl Pharmacol 144:332-339.

Berenson GS, Burch GE (1951) Studies of diffusion through dead human skin. Am J Trop Med Hyg 31:842-853.

Berezhkovskly LM (2004a) Determination of volume of distribution at steady state with complete consideration of the kinetics of protein and tissue binding in linear. J Pharm Sci 93:364-364.

Berezhkovskly LM (2004b) Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. J Pharm Sci 93:1628-1640.

Berezhkovskly LM (2007) The connection between the steady state (Vss) and Terminal (V_β) volumes of distribution in linear pharmacokinetics and the general proof that $V_\beta \geq$ Vss. J Pharm Sci 96:1638-1650.

Bilsback MM, Parker CM, Wimberly HC (1984) Comparative oral toxicity of technical MO70616 and technical SD 42775 in rats. Study No. WRC-RIR 357.Corporation Study No. NCT584.01, Shell Development Company, Houston, TX.

Blancato JN, Knaak JB, Power F (2000) Use of PBPK models for assessing absorbed dose and ChE inhibition from aggregate exposure of infants and children to organophosphorous insecticides. Presented at 10th Annual Meeting of the International Society of Exposure Analysis, Monterey, CA (Abstract 3F-09o).

Bloomquist JR, Soderlund DM (1988) Pyrethroid insecticides and DDT modify alkaloid-dependent sodium channel activation and its enhancement by sea anemone toxin. Mol Pharmacol 33:543-550.

Boatman RJ, Knaak JB (2001) Ethers of ethylene glycol and derivatives. In Patty's Toxicology (5th Edition). Eds Bingham E, Cohrsson B, Powell CH, John Wiley and Sons, Inc. NY, NY.

Bolger MB (2010) Simulations-Plus, Inc., Lancaster, CA. Memo to Kurt Enslein, Enslein Research, Rochester, NY.

Breckenridge CB, Holden L, Sturgess N, Weiner M, Sheets L, Sargent D, Soderlund DM, Choi J-S, Symington S, Clark JM, Burr S, Ray D (2009) Evidence for a separate mechanism of toxicity for the Type I and the Type II pyrethroid insecticides. Neurotoxicology 30.Suppl 1: S17-S31.

Bronaugh RL, Stewart RF, Congdon ER (1982) Methods for in vitro percutaneous absorption studies II. Animal models for human skin. Toxicol Appl Pharmacol 62:481-488.

Bronaugh RL, Stewart RF, Simon M (1986) Methods for in vitro percutaneous absorption studies. VII: use of excised human skin. J Pharma Sci 75:1094-1097.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP (1997) Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13:407-484.

Burr SA, Ray DE (2004) Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicol Sci 77:341-346.

Cahn RS, Ingold CK, Prelog V (1966) Specification of chirality. Angew Chem Int Ed Engl 5:385-415.

Casida JE, Kimmel EC, Elliott M, Janes NF (1971a) Oxidative metabolism of pyrethrins in mammals. Nature 230:326-327.

Casida JE, Kimmel ED, Elliott M, Janes NF (1971b) Oxidative metabolism of pyrethrins in mammals. Pyrethrum Post 11:58-59.

Casida JE, Ueda K, Gaughan LC, Jao LT, Soderlund DM (1975). Structure-biodegradability relationships in pyrethroid insecticides. Arch Environ Contam Toxicol 3:491-500.

Cayley GR, Simpson BW (1986) Separation of pyrethroid enantiomers by chiral high-performance liquid chromatography. J Chromatogr A 356:123-134.

Chamberlain K, Matsuo N, Kaneko H, Khambay BPS (1998) Pyrethroids. In: Chirality in Agrochemicals, Wiley, New York.

Chang SK, Brooks JB, Monteiroriviere NA, Riviere JE (1995) Enhancing or blocking effect of fenvalerate on the subsequent percutaneous absorption of pesticides in vitro. Pest Biochem Physiol 51:214-219.

Chang JH, Benet LZ (2005) Glucuronidation and the transport of the glucuronide metabolites in LLC-PK1 cells. Mol Pharmacol 2:428-434.

Chang DT, Goldsmith M-R, Tornero-Velez L-J, Ulrich E, Lindstrom AB, Dary CC (2009). A novel application of QSAR and PH4 models for the elucidation of the stereoselective hydrolysis rates of pyrethroids by rat serum carboxylesterase. Society of Environmental Toxicology and Chemistry, New Orleans, LA, November 2009 meeting.

Chang DT (2009) F_{up} values derived from Khsa values using QikProp. Unpublished results.

Chemical Abstracts 2002 Index Guide, Appendix IV, Naming and Indexing of Chemical Substances for Chemical Abstracts, E. Stereochemistry and Stereoparents (paragraphs 202-212). Chemical Abstracts Service, Columbus, Ohio.

Choi J-S, Soderlund DM (2006) Structure-activity relationships for the action of 11 pyrethroid insecticides on rat Nav1.8 sodium channels expressed in Xenopus oocytes. Toxicol Appl Pharmacol 211:233-244.

Clark JM, Brooks MW (1989) Role of ion channels and intraterminal calcium homeostasis in the action of deltamethrin at presynaptic nerve terminals. Biochem Pharmacol 38:2233-2245.

Class TJ, Ando T, Casida JE (1990) Pyrethroid metabolism: microsomal oxidase metabolites of (S)-bioallethrin and the six natural pyrethrins. J Agric Food Chem 38:529-537.

Cole LM, Ruzo LO, Wood EJ, Casida JE (1982) Pyrethroid metabolism: Comparative fate in rats of tralomethrin, tralocythrin, deltamethrin, and (1R, alphaS)-*cis*-cypermethrin. J Agric Food Chem 30:631-636.

Colmenarejo G, Alverez-Pedraglio A, Lavandera J (2001) Cheminformatic models to predict binding affinities to human serum albumin. J Med Chem 44:4370-4378.

Colmenarejo G (2003) In silico prediction of drug-binding strengths to human serum albumin. Med Res Rev 23:275-301.

Cramer, RD, Patterson DE, Bunce JD (1988) Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. J Am Chem Soc 110: 5959-5967.

Crane AL, Browner RW, Knaak JB, Bonner MR, Fenske RA, Farahat FM, Anger WK, Lein PJ, Olson JR (2011) Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in human blood following in vitro and in vivo exposure to chlorpyrifos. Abstract No. 1243, 49th Annual Meeting and ToxExpo, Salt Lake City, Utah, March 7-11.

Crawford MJ, Hutson DH (1977) The metabolism of the pyrethroid insecticide (±)-α-cyano-3-phenoxybenzyl 2, 2, 3, 3-tetramethylclyclopropanecarboxylate, WL 41706, in the rat. Pestic Sci 8:579-599.

Crawford MJ, Croucher A, Hutson DH (1981a) Metabolism of *cis*- and *trans*-cypermethrin in rats. Balance and tissue retention study. J Agric Food Chem 29:130-135.

Crawford MJ, Croucher A, Hutson DH (1981b) The metabolism of the pyrethroid insecticide cypermethrin in rats: Excreted metabolites. Pestic Sci 12:399-411.

Crow JA, Borazjani A, Potter PM, Ross MK (2007) Hydrolysis of pyrethroids by human and rat tissues: examination of intestinal, liver and serum carboxylesterases. Toxicol Appl Pharmacol 221:1-12.

Culliford SJ, Borg JJ, O'Brien MJ, Kozlowski RZ (2004) Differential effects of pyrethroids on volume-sensitive anion and organic osmolyte pathways. Clin Exp Pharmacol Physiol 31:134-144.

Danker T, Mazzanti M, Tonini R, Rakowska A, Oberleithner H (1997) Using the atomic force microscopy to investigate patch-clamped nuclear membrane. Cell Biology International 21:747-757.

Davies B, Morris T (1993) Physiological parameters in laboratory animals and humans. Pharm Res (NY) 10:1093-1095.

Delie F, Rubas W (1997) A human colonic cell line sharing similarities with enterocytes as a model to examine oral absorption: Advantages and limitations of the Caco-2 model. Crit Rev Ther Drug Carrier Syst 14:221-286.

Diel F, Detscher M, Schock B, Ennis M (1998) In vitro effects of the pyrethroid S-bioallethrin on lymphocytes and basophils from atopic and nonatopic subjects. Allergy 53:1052-1059.

Deltamethrin Monograph (1982), J. L'Hoste (ed) Deltamethrin Monograph, Roussel-Uclaf, Paris.

Eadsforth CV, Baldwin MK (1983) Human dose-excretion studies with pyrethroid insecticide, cypermethrin. Xenobiotica 13:67-72.

Eadsforth CV, Bragt PC, Sittert NJ (1988) Human dose-excretion studies with pyrethroid insecticides: cypermethrin and alpha cypermethrin: relevance for biological monitoring. Xenobiotica 18:603-614.

EC-HCPDG (2002) European Commission, Health & Consumer Protection Directorate-General, Directorate E1: Plant Health. Beta-Cyfluthrin, Review Report # 6841/VI/97-final, 12-2-2002.

Eells JT, Bandettini PA, Holman PA, Propp JM (1992) Pyrethroid insecticide-induced alterations in mammalian synaptic membrane-potential. J Pharmacol Exp Ther 262:1173-1181.

Eldefrawi ME, Sherby SM, Abalis IM, Eldefrawi AT (1985) Interactions of pyrethroid and cyclodiene insecticides with nicotinic acetylcholine and GABA receptors. Neurotoxicology 6:47-62.

Elflein L, Berger-Preiss E, Preiss A, Elend M, Levsen K, Wunsch G (2003) Human biomonitoring of pyrethroid insecticides used indoors: determination of the metabolites E-*cis/trans*-chrysanthemum dicarboxylic acid in human urine by gas chromatography-mass spectrometry with negative chemical ionizations. J Chromatogr B 795:195-207.

Elliott M, Farnham AW, Janes NF, Needham PH, Pearson BC (1967) 5-Benzyl-3-furylmethyl chrysanthemate a new potent insecticide. Nature (London) 213:493-494.

Elliott M, Janes NF, Kimmel EC, Casida JE (1972) Metabolic fate of pyrethrin I, pyrethrin II, and allethrin administered orally to rats. J Agric Food Chem 20:300-313.

Elliott M, Farnham AW, Janes NF, Needhan DH, Pulman DA (1974) Synthetic insecticides with a new order of activity. Nature (London) 248:710-711.

Elliott M, Janes NF, Pulman DA, Gaughan LC, Unai T, Casida JE (1976) Radiosynthesis and metabolism in rats of the 1R-isomers of the insecticide permethrin. J Agric Food Chem 24:270-276.

Elliott M, Janes NF, Potter C (1978) The future of pyrethroids in insect control. Ann Rev Entomol 23:443-469.

Elliott M, Janes NF (1978) Synthetic pyrethroids: A new class of insecticide. Chem Soc Rev 7:473-505.

Elliott M (1989) The Pyrethroids: Early discovery, recent advances and the future. Pestic Sci 27:337-351.

Ellison CA, Knaak, JB, McDougall R, Lein PJ, Farahat FM, Anger WK, Olson JR (2011) Construction and validation of a human PBPK/PD model for dermal chlorpyrifos exposure utilizing human biomarker data. Abstract No. 2106, 50th Annual Meeting and ToxExpos, Washington, DC, March 6-10.

Enan E, Matsumura F (1993) Activation of phosphoinositide protein-kinase-c pathway in rat-brain tissue by pyrethroids. Biochem Pharmacol 45:703-710.

Enslein K, Gombar VK, Shapiro D, Blake BW (1998) Prediction of rat oral LD_{50} values of chemicals by QSAR equations. TOPKAT Health Designs, Rochester, NY.

Enslein K, Knaak JB, Van Nostrand K, Susa R, Koestler D (2007) CYP3A4 hydroxylation kinetics: QSAR models with application to PBPK modeling. J CompAided Mol Design (submitted).

Enslein K (2010) ADMET Predictor, Simulations-Plus, Lancaster, CA.

Farahat FM (2009) (personal communications).

Farahat FM, Ellison CA, Bonner MR, McGarrigle BP, Crane AL, Fenske RA, Lasarev MR. Rohlman DS, Anger WK, Lein PJ, Olson JR (2011). Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers. Environ Health Perspect 119: 801-816.

Farahmand S, Maibach HI (2009) Estimating skin permeability from physicochemical characteristics of drugs: A comparison between conventional models and an in vivo-based approach. Int J Pharm 375:41-47.

Faraoni M, Messina A, Polcaro CM, Aturki Z, Sinibaldi M (2004) Chiral separation of pesticides by coupled-column liquid chromatography application to the stereoselective degradation of fenvalerate in soil. J Liq Chromatogr Rel Technol 27: 995-1012.

Feldmann RJ, Maibach HI (1974) Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol 28:126-132.

Fisher JB, Debray PH, Robinson J (1983) In: Plant Protection for Human Welfare, Proc 10th Internat Congr Plant Prot Vol 1, BCPC, Croydon, UK, 1983, pp 452-459. FMC, U.S. Patent 4 262 921 (1981).

Fleischer R (1877) Untersuchgen uber das resporptions-vermogen der menschlichen haut. Erlangen 1877. Habilitationsschrift p 81.

Flynn GL (1990) Physicochemical determinants of skin absorption. In: Principles of Route-to-Route Extrapolation for Risk Assessment, TR Gerrity and CJ Henry (eds.), Elsevier, New York, pp 93-127.

Foxenberg RJ, Knaak JB, McGarrigle BP, Kostyniak PJ, Olson JR (2007) Human hepatic cytochrome P450-specific metabolism of parathion and chlorpyrifos. Drug Metab Dispos 35:189-193.

Foxenberg RJ, Ellison CA, Knaak JB, Ma C, Olson JR (2011) Cytochrome P450-specific human PBPK/PD models for the organophosphorus pesticides: chlorpyrifos and parathion. Toxicology 285:57-66.

Freeman C (1982) Acute oral toxicity study in rats: FMC 54800. Study No. A83-859, FMC Corporation, Philadelphi PA.

Freeman C (1987) Acute oral toxicity study-FMC 45806 technical: Study No. A87-2282, FMC Corporation, Philadelphia, PA.

Freeman C (1989) Acute oral toxicity of FMC 56701 technical in rats. Study No. A89-2914, FMC Corporation, Philadelphia, PA.

Fujikawa M, Nakao K, Shimizu R, Akamatsu M (2007) QSAR study on permeability of hydrophobic compounds with artificial membranes. Bioorg Med Chem 15:3756-3767.

Fujimoto K, Itaya N, Okuno Y, Kadota T, Yamaguchi T (1973) A new insecticidal pyrethroid ester. Agric Biol Chem 37:2681-2682.

Furnax R, Audegond L (1985a) Bioallethrin (synthetic) acute oral toxicity in rat. Study No. 84324/5635/5637, Roussel-Uclaf, Paris.

Furnax R, Audegond L (1985b) RU 16121 acute oral toxicity in rat. Study No. 84327/5640/5641, Roussel-Uclaf, Paris.

Gabriel D (1992) Acute oral toxicity study in rats. Study No. 92-7529A, Pyrethrin Joint Venture Chemical Specialties Manufacturing Association, Washington, DC.

Gao R, Zhu L, Chen Z (1998) Separation of pyrethroid enantiomers of fenpropathrin and fluvalinate by chiral high performance liquid chromatography. Nongyao (Pesticides) 37(9):22-24.

Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME (1989) Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98:87-99.

Gassner B, Wuthrich A, Scholtysik G, Solioz M (1997) The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. J Pharmacol Exp Therap 281:855-860.

GastroPlus Users Manual, version 6.0, Simulations-Plus, Inc., Lancaster, CA

Gaughan LC, Unai T, Casida JE (1977) Permethrin metabolism in rats. J Agric Food Chem 25:9-17.

Geinoz S, Guy RH, Testa B, Carrupt PA (2004) Quantitative structure-permeation relationships (QSPeRs) to predict skin permeation: a critical evaluation. Pharm Res 21:83-92.

Ghiasuddin SM, Soderlund DM (1985) Pyrethroid insecticides potent, stereospecific enhancers of mouse brain sodium channel activation. Pestic Biochem Physiol 24:200-206.

Girelli AM, Messina A, Sinibaldi M (2002) A study on the separation of synthetic pyrethroid stereoisomers by HPLC. Ann Chim 92:417-424.

Glomot R (1979) RU 25474, active material: acute oral toxicity study in the rat. American Hoechst Corporation Study No. RU-4BE-79818-19/A.

Godin SJ, Scollon EJ, Hughes MF, Potter PM, DeVito MJ, 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, 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.

Godin SJ, DeVito MJ, Hughes MF, Ross DG, Scollon EJ, Starr JM, Setzer RW, Conolly RB, Tornero-Velez, R (2010) Physiologically based pharmacokinetic modeling of deltamethrin: development of a rat and human diffusion-limited model. Toxicol Sci 115: 330-343.

Goksu EI, Vanegas JM, Blanchette CD, Lin W-C, Longo ML (2009) AFM for structure and dynamics of biomembranes. Biochemica et Biophysica Acta 1788:254-266.

Goodman LS (2001) Goodman & Gilman's the pharmacological basis of therapeutics. 10th ed. / New York: McGraw-Hill Medical Pub. Division.

Gray AJ, Soderlund DM (1985) Mammalian toxicology of pyrethroids, In: Hutson DH, Roberts TR (eds), Insecticides, Wiley, New York, 1985, pp 193-248.

Grosman N, Diel F (2005) Influence of pyrethroids and piperonyl butoxide on the Ca^{+2} – ATPase activity. Int J Immunopharmacol 5:263-270.

Guengerich FP, Martin MV (1998) Purification of cytochromes P450. Rat and human hepatic forms. Methods Mol Biol 107:35-53.

Guy RH, Hadgraft J, Bucks DA (1987) Transdermal drug delivery and cutaneous metabolism, Xenobiotica; Fate Foreign Compd Biol Syst 17:325-343.

Hagiwara N, Irisawa H, Kameyama M (1988) Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol 395:233-253.

Hallifax D, Houston JB (2006) Binding of drugs to hepatic microsomes: comment and assessment of current prediction methodology with recommendation for improvement. Drug Metab Dispos 34:724-726.

Hamman I, Fuchs R (1981) Baythroid®, a new insecticide. Pflanzenschutz-Nachrichten Bayer 34:121-151.

Hansch C, Hoekman D, Leo A, Zhang L, Li P (1995) The expanding role of quantitative structure-activity relationships (QSAR) in toxicology. Toxicol Lett 79:45-43.

Hardt J, Angerer J (2003) Biological monitoring of workers after the application of insecticidal pyrethroids. Int Arch Occup Environ Health 76:492-498.

Harrison SM, Barry BW, Dugard PH (1984) Effects of freezing on human-skin permeability. J Pharm Pharmacol 36:261-262.

Heimann KG (1987) FCR 1272 (c.n. cyfluthrin): Study for acute oral toxicity to rats (formulation acetone and peanut oil). Bayer Corporation Agricultural Division Report No. 106805.

Hemming H, Flodstrom S, Warngard L (1993) Enhancement of altered hepatic foci in rat-liver and inhibition of intercellular communication in-vitro by the pyrethroid insecticides fenvalerate, flucythrinate and cypermethrin. Carcinogenesis 14:2531-2535.

Henrick CA, Garcia BA, Staal GB, Cerf DC, Anderson RJ, Gill K, Chinn HR, Labovitz JN, Leippe MM, Woo SL, Carney RL, Gordon DC, Kohn GK (1980) 2-Anilino-3-methylbutyrates and 2-(isoindolin-2-yl)-3-methylbutyrates, two novel groups of synthetic pyrethroid esters not containing a cyclopropane ring. Pestic Sci 11(2):224-241.

Henrick CA (1977) Zoecon Corp. (patent for fluvalinate development).

Hildebrand ME, McRory JE, Snutch TP, Stea A (2004) Mammalian voltage-gated calcium channels are potently blocked by the pyrethroid insecticide allethrin. J Pharmacol Exp Therap 308:805-813.

Hiromori T, Misaki Y, Seki T, Hosokawa S, Miyamoto J (1983) Acute oral toxicity of S-3206 (91.8%) in rats. Sumitomo Chemical America (unpublished study).

Hood SR, Shah G, Jones P (1998) Expression of cytochromes P450 in a Baculovirus system. In: Phillips IR, Shepard EA (eds) Methods in Molecular Biology: CYP Protocols. Humana Press, Totowa, NJ, Ch 24, pp 203-218.

Horber JKH, Mosbacher J, Haberle W, Ruppersberg JP, Sakmann B (1995) A look at membrane patches with a scanning force microscope. Biophysical Journal 68:1687-1693.

Hosokawa M, Maki T, Satoh T (1987) Muliplicity and regulation of hepatic microsomal carboxylesterases in rats. Mol Pharmacol 31:579-584.

Hosokawa M, Maki T, Satoh T (1990) Characterization of molecular species of liver microsomal carboxylesterases of several animal species and humans. Arch Biophys 277:219-227.

Hotchkiss SAM, Miller JM, Caldwell J (1992) Percutaneous absorption of benzyl acetate through rat skin in vitro. 2. Effect of vehicle and occlusion. Food Chem Toxicol 30:145-153.

Houston JB (1994) Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. Biochem Pharmacol 47:1469-1479.

Huang T, Kuang C, Zhou J, Gou D (1991) Chiral separation of water soluble. BETA-Lactam Enanatiomers on BETA-Cyclodextrin bonded stationary phase. Fenxi Huaxue 16:687-689.

Huckle KR, Chipman JK, Hutson DH, Miliburn P (1981a) Metabolism of 3-phenoxybenzoic acid and the enterohepatorenal disposition of its metabolites in the rat. Drug Metab Dispos 9:360-368.

Huckle KR, Hutson DH, Millburn P (1981b) Species differences in the metabolism of 3-phenoxybenzoic acid. Drug Metab Dispos 9:352-359.

Huckle KR, Hutson DH, Millburn P (1984) Metabolism of 3-phenoxybenzoic acid in isolated rat hepatocytes and renal tubule fragments. Drug Metab Dispos 12:264-265.

Huff RK (1978) Halogenated esters of cyclopropane acids, their preparation, composition and use as pesticides. Eur Pat 0010879 ICI.

Hutson DH, Logan CJ (1986) The metabolic fate in rats of the pyrethroid insecticide WL85871, a mixture of two isomers of cypermethrin. Pestic Sci 17:548-558.

IARC, Occupational Exposures in Insecticide Application and Some Pesticides, IARC, Lyon, 1991.

Inoue S, Howgate EM, Rowland-Yeo K, Shimada T, Yamazaki H, Tucker GT, Rostami-Hodjegan A (2006) Prediction of in vivo drug clearance from in vitro data. II: Potential inter-ethnic differences. Xenobiotica 36:499-513.

Irvine JD, Takahashi L, Lockhart K, Cheong J, Tolan JW, Selick HE, Grove JR (1999) MDCK (Madin-Darby Canine Kidney) Cells: A tool for membrane permeability screening. J Pharm Sci 88:28-33.

Iwatsubo T, Hirota N, Ooie T, Suzuki H, Chiba K, Ishizaki T, Green CE, Tyson CA, Sugiyama Y (1997) Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. Pharmacol Ther 73:147-171.

Izumi T, Kaneko H, Matsuo M, Miyamoto J (1984) Comparative metabolism of the six stereoisomers of phenothrin in rats and mice. J Pestic Sci 9:259-267.

Jarabek A (2002) External Review Draft NCEA-1-0503, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

Jepson GW, Hoover DK, Black RK, McCafferty JD, Mahle DA, Gearhart JM (1992) Partition coefficient determination for nonvolatile and intermediate volatility chemicals in biological tissues. Toxicology 12:262.

Jepson GW, Hoover DK, Black RK, McCafferty JD, Mahle DA, Gearhart JM (1994) A partition coefficient determination methods for non-volatile chemicals in biological issues. Fundam Appl Toxicol 22:519-524.

Johnson PL (2007) Synthesis of (S)-cyano (3-phenoxyphenyl) methyl (1R, 3R)-3-[(1Z)-2-chloro-3, 3, 3-trifluoro-1-propenyl)-2, 2-dimethylcyclopropanecarboxylate-1-^{14}C. J Label Compd Radiopharm 50:47-53.

Jutsum AR, Gordon RF, Ruscoe CNE (1986) In: Proc. 1986 Brit Crop Prot Conf Pests and Diseases, BCPC, Croydon, pp 97-106.

Kakko I, Toimela T, Tahti H (2003) The synaptosomal membrane bound ATPase as a target for the neurotoxic effects of pyrethroids, permethrin and cypermethrin. Chemosphere 51:475-480.

Kaneko H, Ohkawa H, Miyamoto J (1981a) Adsorption and metabolism of dermally applied phenothrin in rats. Nippon Noyaku Gakkaishi 6:169-182.

Kaneko H, Ohkawa H, Miyamoto J (1981b) Comparative metabolism of fenvalerate and the [2S, αS]-isomer in rats and mice. Nippon Noyaku Gakkaishi 6:317-326.

Kaneko H, Ohkawa H, Miyamoto J (1981c) Metabolism of tetramethrin isomers in rats. Nippon Noyaku Gakkaishi 6:425-435.

Kaneko H, Matsuo M, Miyamoto J (1984) Comparative metabolism of stereoisomers of cyphenothrin and phenothrin isomers in rats. Nippon Noyaku Gakkaishi 9:237-247.

Kaneko H, Shiba K, Yoshitake A, Miyamoto J (1987) Metabolism of fenpropathrin (S-3206) in rats. Nippon Noyaku Gakkaishi 12:385-395.

Kaneko H, Miyamoto J (2001) Pyrethroid chemistry and metabolism, Chapter 58, In: Hayes Handbook of Pesticide Toxicology, 2nd Ed, (ed) Krieger R , Academic Press, San Diego, CA.

Kaneko H (2010) Pyrethroid Chemistry and Metabolism, Chapter 76. In: Hayes' Handbook of Pesticide Toxicology, 3rd Edition, (ed) Krieger R, Academic Press, San Diego, CA.

Kansy M, Senner F, Gubernator K (1998) Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. J Med Chem 41(7):1007-1010.

Kato T, Ueda K, Fujimoto K (1965) New insecticidally active chrysanthemate. Agric Biol Chem 28:914-915.

Katsuda Y (1975) Sumitomo Chemical Co, LTD. (patent for fluvalinate development).

Katsuda Y (1999) Development of and future prospects for pyrethroid chemistry. Pestic Sci 55:775-782.

Khambay BPS (2002) Pyrethroid insecticides. Royal Society of Chemistry (UK). Pestic Outlook 49-54.

Killeen JCJ (1975) Acute oral toxicity study in rats with FMC 33297, FMC Corp, Philadelphia, PA.

Klopman G, Rosenkranz HS (1995) Toxicity estimation by chemical substructure analysis: the TOX II program. Toxicol Lett 79:145-155.

Knaak JB, Iwata Y, Maddy KT (1989) The worker hazard posed by reentry into pesticide-treated foliage: development of safe reentry times, with emphasis on chlorthiophos and carbosulfan. In: Paustenbach D (ed) The Risk Assessment of Environmental Hazards: A Textbook of Case Studies. Wiley, New York, Ch 24, pp 797-842.

Knaak JB, Wagner B, Boutchyard Jr. H, Smith LW, Jones T, Wang RH (1993) Computerization of toxicological data by government agencies, chemical, and information industries. In: Jolley RL and Wang RGM (eds) Effective and Safe Waste Management; Interfacing Sciences and Engineering with Monitoring and Risk Analysis, Lewis Publishers, Boca Raton, Florida, Ch 19, pp 227-264.

Knaak JB, Leung H-W, Stott WT, Busch J, Bilsky J (1997) Toxicology of Mono-, Di-, and Triethanolamine. Rev Environ Contam Toxicol 149:1-86.

Knaak JB, Dary C, Patterson GT, Blancato J (2002) The worker hazard posed by reentry into pesticide-treated foliage: reassessment of reentry levels/intervals using foliar residue transfer-percutaneous absorption PBPK/PD models, with emphasis on isofenphos and parathion. In: Paustenbach D (ed) Human and Ecological Risk Assessment: Theory and Practice. Wiley, New York, pp 673-731.

Knaak JB, Dary CC, Power F, Thompson CB, Blancato JN (2004) Physicochemical and biological data for the development of predictive organophosphorus pesticides QSARs and PBPK/PD models for human risk assessment. Crit Rev Toxicol 34:143-207.

Knaak JB, Dary CC, Okino MS, Power FW, Zhang X, Thompson CB, Tornero-Velez R, Blancato JN (2008) Parameters for carbamate pesticide QSAR and PBPK/PD models for human risk assessment. Rev Environ Contam Toxicol 193:53-212.

Kuo JK (1985) Phospholipids and Cellular Regulation. CRC Press, Boca Raton, FL.

Kurihara N, Miyamoto J, Paulson GD, Zeeh B, Skidmore MW, Hollingsworth RM, Kuiper HA (1997a) Chirality in synthetic agrochemicals: bioactivity and safety considerations. Pure & Appl Chem 69:1335-1348.

Kurihara N, Miyamoto J, Paulson GD, Zeeh B, Skidmore MW, Hollingsworth RM, Kipper HA (1997b) Chirality in synthetic agrochemicals: Bioactivity and safety consideration. Pure & Appl Chem 69:2007-2026.

Kutter JP, Class TJ (1992) Diastereoselective and enantioselective chromatography of the pyrethroid insecticides allethrin and cypermethrin. Chromatograhia 33: 103-112.

LaForge FB, Haller HL (1936) Constituents of pyrethrum flowers. VI. The structure of pyrethrolone. J Am Chem Soc 58:1777-1780.

LaForge FB, Soloway SB (1947) Structure of dihydrocinerolone. J Am Chem Soc 69:186.

Lau YY, Wu C-Y, Okochi H, Benet LZ (2003) Ex situ inhibition of hepatic uptake and efflux significantly changes metabolism: hepatic enzyme-transporter interplay. J Pharmacol Exp Ther 308:1040-1045.

Lawrence LJ, Gee KW, Yamamura HI (1985) Interactions of pyrethroid insecticides with chloride ionophore-associated binding sites. Neurotoxicology 6:87-98.

Lee W, Kim B-H (1998) Liquid chromatographic resolution of pyrethroic acids and their esters on chiral stationary phases. J High Res Chromatog 21:189-192.

Leng G, Leng A, Kuhn K.-H, Lewalter J, Pauluhn J (1997) Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: urinary metabolite profile following inhalation. Xenobiotica 27:1273-1293.

Li Z-Y, Zhang Z-C, Zhou Q-L, Wang Q-M, Gao R-Y, Wang Q-S (2003) Stereo and enantioselective determination of pesticides in soil by using achiral and chiral liquid chromatography in combination with matrix solid-phase dispersion. J AOAC Int 86(3):521-528.

Li Z-Y, Zhang Z-C, Zhang L, Leng L (2006) Chiral separation of pyrethroids pesticides and fenvaleric acid. Fenxi Shiyanshi (Chinese Journal of Analysis Laboratory) 25(11):11-14.

Li Z, Zhang Z, Zhang L, Leng L (2009) Isomer- and enantioselective degradation and chiral stability of fenpropathrin and fenvalerate in soils. Chemosphere 76:509-516.

Liang R, Fei Y-J, Prasad PD, Ramamoorthy S, Han H, Yang-Feng TL, Hediger MA, Ganapathy V, Leibach FH (1995) Human intestinal H^+/peptide cotransporter: Cloning, functional expression, and chromosomal localization. J Biol Chem 270:6456-6463.

Lin JH, Yamazaki M (2003) Role of P-glycoprotein in pharmacokinetics: Clinical Implications. Clin Pharmacokinet 42:59-98.

Lipscomb JC, Fisher JW, Confer PD, Byczkowski JZ (1998) In vitro to in vivo extrapolation for trichloroethylene metabolism in humans. Toxicol Appl Pharmacol 152:376-387.

Liu HX, Yao XJ, Zhang RS, Liu MC, Hu ZD, Fan BT (2005) Prediction of the tissue /blood partition coefficients of organic compounds based on the molecular structure using least-squares support vector machines. J Comput-Aided Mol Design 19:499-508.

Liu W, Gan J, Schlenk D, Jury WA (2005a) Enantioselectivity in environmental safety of current chiral insecticides. Proc Natl Acad Sci USA. 102(3):701-706.

Liu W, Gan JJ. Qin S (2005b) Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. Chirality 17:S127-S133.

Liu W, Gan J, Lee S, Werner I (2005c) Isomer selectivity in aquatic toxicity and biodegradation of bifenthrin and permethrin. Environ. Toxicol. Chem. 24(8):1861-1866.

Lu AYH, West SB (1980) Multiplicity of mammalian microsomal cytochrome P450. Pharmacol Rev 31:277-291.

Maibach HI, Feldmann RJ, Milby TH, Serat WF (1971) Regional variation in percutaneous penetration in man. Arch Environ Health 23:208-211.

Mancini F, Fiori J, Bertucci C, Cavrini V, Bragieri M, Zanotti MC, Liverani A, Borzatta V, Andrisano V (2004) Stereoselective determination of allethrin by two-dimensional achiral/chiral liquid chromatography with ultraviolet/circular dichroism detection. J Chromatogr A, 1046:67-73.

Martel J (1976) Roussel UCLAF (patent, tralomethrin development).

Matsuo T, Itaya N, Mizutani T, Ohno N, Fujimoto K, Okuno Y, Yoshioka H (1976) 3-phenoxy-a-cyanobenzyl esters, the most potent synthetic pyrethroids. Agric Biol Chem 40:247-249.

Mattie DR, Bates GD, Jepson GW, Fisher JW, McDougal JN (1994) Determination of skin: air partition coefficients for volatile chemicals: experimental method and applications. Toxicol Appl Pharmacol 22:51-57.

McGregor DB (1999) Permethrin Joint Meeting, FAO Panel of Experts on Pesticide Residues in Food and the Environment, and WHO Core Assessment Group, Rome.

Meylan WM, Howard PH (1994a) Upgrade of PCGEMS water solubility estimation method (draft). U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC.

Meylan WM, Howard PH (1994b) Validation of water solubility estimation methods using log K_{ow} for application in PCGEMS & EPI. Final report, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC.

Mirfazaelian A, Kim K-B, Anand SS, Kim HJ, Tornero-Velez R, Bruckner JV, Fisher JW (2006) Development of a physiologically based pharmacokinetic model for deltamethrin in the adult male Sprague-Dawley rat. Toxicol Sci 93:432-442.

Miyamoto J, Nishida T, Ueda K (1971) Metabolic fate of resmethrin, 5-benzyl-3-furylmethyl dl-*trans* chrysanthemate in the rat. Pestic Biochem Physiol 1:293-306.

Miyamoto J, Suzuki T, Nakae C (1974) Metabolism of phenothrin (3-phenoxybenzyl d-*trans*-chrysanthemumate) in mammals. Pestic Biochem Physiol 4:438-450.

Miyamoto J (1976) Degradation, metabolism and toxicity of synthetic pyrethroids. Environ Health Persp 14:15-28.

Moss GP, Derden JC, Patel H, Cronin MT (2002) Quantitative structure-permeability relationships (QSPRs) for percutaneous absorption. Toxicol In Vitro 16:299-317.

Mugeng J, Soderlund DM (1982). Liquid chromatographic determination and resolution of the enantiomers of the acid moieties of pyrethroid insecticides as their (-)-1-(1-phenyl)ethylamide derivatives. J Chromatogr A. 248(1):143-149.

Myer JR (1989) Acute oral toxicity study of deltamethrin in rats. Hoechst-Roussel Agri-Vet Company Study No. 327-122.

Nakamura Y, Sugihara K, Sone T, Isobe M, Ohta S, Kitamura S (2007) The in vitro metabolism of a pyrethroid insecticide, permethrin, and its hydrolysis products in rats. Toxicology 235:176-184.

Narahashi T (1971) Mode of action of pyrethroids. Bull World Health Organ 44:337-345.

Nardini M, Dijkstra BW (1999) α/β Hydrolase fold enzymes: the family keeps growing, Current Opinion in Structural Biology 9: 732-737.

Naumann K (1998) Research into fluorinated pyrethroid alcohols-an episode in the history of pyrethroid discovery. Pestic Sci 52:3-20.

Nicoli S, Santi P (2007) Suitability of excised rabbit ear skin – fresh and frozen – for evaluation Transdermal permeation of estradiol. Drug Deliv 14:195-199.

Nigg HN, Knaak JB (2000) Blood cholinesterases as human biomarkers of organophosphorus pesticide exposure. Rev Environ Contam Toxicol 163:29-112.

Nikolelis DP, Brennan JD, Brown S, McGibbon G, Krull UJ (1991) Ion permeability through bilayer lipid membranes for biosensor development: Control by chemical modification of interfacial regions between phase domains. Analyst 116:1221-1226.

Nishizawa Y (1971). Development of New Synthetic Pyrethroids. Bull World Health Organ 44:325-336.

Nong A, Tan Y-M, Krolski ME, Wang J, Lunchick C, Conolly RB, Clewell III HJ (2008) Bayesian calibration of a physiologically-based pharmacokinetic/pharmacodynamic model of carbaryl cholinesterase inhibition. J Toxicol Environ Health, Part A 71: 1363-1381.

Obach RS (1996) The importance of nonspecific binding in in vitro matrices, its impact on enzyme kinetic studies of drug metabolism and implications for in vitro-in vivo correlations. Drug Metab Dispos 24:1047-1049.

Obach RS (1997) Nonspecific binding to microsomes: impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol. Drug Metab Dispos 25:1359-1369.

Obach RS (1999) The prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of the in vitro half-life approach and non-specific binding to microsomes. Drug Metab Dispos 27:1350-1359.

Ohkawa H, Kaneko H, Tsuji H, Miyamoto J (1979) Metabolism of fenvalerate (Sumicidin) in rats. J Pestic Sci (J Nippon Noyaku Gakkaishi) 4:143-155.

Ohno N, Fujimoto K, Okuno Y, Mizutani T, Hirano M, Yoshioka H (1974) A new class of pyrethroidal insecticides: α-substituted phenylacetic acid esters. Agric Biol Chem 38:881-883.

Oi N, Horiba M, Kitahara H (1981) Gas chromatographic separation of optical isomers of chrysanthemic acid on an optically active stationary phase. Agric Biol Chem 45(6):1509-1510.

Oi N, Kitahara H, Kira R (1990) Enantiomer separation of pyrethroid insecticides by high-performance liquid chromatography with chiral stationary phases. J Chromatogr A 515:441-450.

Oi N, Kitahara H, Aoki F, Kisu N (1995) Direct separation of carboxylic acid enantiomers by high-performance liquid chromatography with amide and urea derivatives bonded to silica gel as chiral stationary phases. J Chromatogr A 689(2):195-201.

Oi N, Kitahara H, Matsushita Y, Kisu N (1996) Enantiomer separation by gas and high-performance liquid chromatography with tripeptide derivatives as chiral stationary phases. J Chromatogr A 722(1-2):229-232.

Oi N (2005) Development of practical chiral stationary phases for chromatography and their applications. Chromatography, 26(1):1-5.

Omura T, Sato (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 239:2370-2378.

O'Reilly AO, Khanbay BPS, Williamson MS, Field LM, Wallace BS, Davies TGE (2006) Modeling insecticide-binding sites in the voltage-gated sodium channel. Biochem J 396:255-263.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KOL, Marsh Cl, Perkins JD, Thummel KE (1997) Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. J Pharmacol Exp Therap 283:1552-1562.

Papadopoulou-Mourkidou E (1985) Direct analysis of fenvalerate isomers by liquid chromatography. Application to formulation and residue analysis of fenvalerate. Chromatographia 20(6):376-378.

Payne MP, Kenny LC (2002) Comparison of models for the estimation of biological partition coefficients. J Toxicol Environ Health, Part A 65:897-931.

Plummer EL, Cardis AB, Martinez AJ, Van Saun WA, Palmere RM, Pincus DS, Stewart RR (1983) Pyrethroid insecticides derived from substituted biphenyl-3-methods. Pestic Sci 14:560-570.

PMRA (Canada Pest Management Regulatory Agency) (2005) Report PRDD#2005-02 (imiprothrin), www.pmra-arla.gc.ca/english/pdf/prdd/prdd2005-02-e.pdf, Aug 8 2005.

Porcelli C, Roncaglioni A, Chana A, Boriani E, Benfenati A (2007) A protocol for quantitative structure, activity relationship (QSAR) for regulatory purposes: The example of DEMETRA. In Environmental Fate and Ecological Effects of Pesticides, Symposium Pesticide Chemistry, 13th, Placenza, Italy, Sept. 3-6, 2007, 669-674.

Potts RO, Guy RH (1992) Predicting skin permeability. Pharm Res 9:663-669.

Potts RO, Guy RH (1995) A predictive algorithm for skin permeability: the effects of molecular size and hydrogen bond activity. Pharm Res 12:1628-1633.

Poulin P, Theil F-P (2000) A priori prediction of tissue: plasma partition coefficients of drugs to facilitate the use of physiologically based pharmacokinetic models in drug discovery. J Pharm Sci 89:16-35.

Poulin P, Theil F-P (2002a) Prediction of pharmacokinetics prior to in vivo studies. I. Mechanism-based prediction of volume of distribution. J Pharm Sci 91:129-156.

Poulin P, Theil F-P (2002b) Prediction of pharmacokinetics prior to in vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition. J Pharm Sci 91:1358-1370.

Prout M, Howard E, Soames A (1985a) PP993: Absorption, excretion and tissue distribution of a single oral dose (1 mg/kg) in the rat. Syngenta unpublished report, no. CTL/P/1064, UK.

Prout M, Howard E (1985b) PP993: Excretion and tissue distribution of a single oral dose (10 mg/kg) in the rat. Syngenta unpublished report, no. CTL/P/1256, UK.

Prout M, Gibson N, Howard E (1986) PP993: Biotransformation in the rat. Syngenta unpublished report, no. CTL/P/1295, UK.

Pyrethrum: The Natural Insecticide, (ed) Casida JE, Academic Press, New York and London, 1973.

Quistad GB, Staiger LE, Jamieson GC, Schooley DA (1983) Fluvalinate metabolism by rats. J Agric Food Chem 31:589-596.

Rand G (1983) Acute oral toxicity-cypermethrin technical. FMC Corporation Study No. A82-727.

Rao RN, Shankaraiah B, Sunder MS (2004) Separation and determination of diastereomers of γ-Cyhalothrin by normal phase-liquid chromatography using a CN column. Anal Sci 20:1745-1748.

Ray DE, Sutharsan S, Forshaw PJ (1997) Actions of pyrethroid insecticides on voltage-gated chloride channels in neuroblastoma cells. Neurotoxicology 18:755-760.

Ray DE, Fry JR (2006) A reassessment of the neurotoxicity of pyrethroids insecticides. Pharmacol & Therap 111:174-193.

Reifenrath, WG, Kamppainen BW (1991) Skin storage conditions. In: Bronaugh R, Maibach H (eds.), In Vitro Percutaneous Absorption: Principles, Fundamentals and Applications. CRC, Boca Raton, pp 115-125.

Reifenrath WG, Ross JH, Driver JH (2011) Experimental methods for determining permethrin dermal absorption. Submitted for review and publication.

Rekling JC, Theophilidis GN (1995) Effects of the pyrethroid insecticide, deltamethrin, on respiratory modulated hypoglossal moto-neurons in a brain stem slice from newborn mice. Neurosci Lett 198:189-192.

Riddles PW, Richards LJ, Bowles MR, Pond SM (1991) Cloning and analysis of a cDNA encoding a human liver carboxylesterase. Gene (Amst) 108:389-392.

Riviere JE, Bowman KF, Monteiro-Riviere NA, Dix LP, Carver MP (1986) The isolated perfused porcine skin flap (IPPSF). I. A novel in vitro model for percutaneous absorption and cutaneous toxicology studies. Fund Appl Toxicol 7:444-453.

Riviere JE, Monteiro-Riviere NA (1991) The isolated perfused porcine skin flap as an in vitro model for percutaneous absorption and cutaneous toxicology. Crit Rev Toxicol 21:329-344.

Robey RW, Lin B, Qui J, Chan LL, Bates SE (2010) Rapid detection of ABC transporter interaction: potential utility in pharmacology. J Pharmacol Toxicol Methods 63:217-222.

Rodgers T, Leahy D, Rowland M (2005) Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci 94:1259-1276.

Rodgers T, Leahy D, Rowland M (2007) (ERRATA) Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate to strong bases. J Pharm Sci 94:1259-1276. J Pharm Sci 96:3151-3152.

Rodgers T, Rowland M (2006) Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutral and zwitterions. J Pharm Sci 95:1238-1257.

Rodgers T, Rowland M (2007) Mechanistic approaches to volume of distribution predictions: understanding the processes. Pharm Res 24:918-933.

Rodrigues AD (1999) Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochrome P450 and native human liver microsomes. Biochem Pharmacol 57:465-480.

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

Rowland M (2010) Personal communication.

Ruzo LO, Casida JE (1977) Metabolism and toxicology of pyrethroids with dihalovinyl substituents. Environ Health Perspectives 21:285-292.

Ruzo LO, Unai T, Casida JE (1978) Decamethrin metabolism in rats. J Agric Food Chem 26:918-925.

Ruzo LO, Engel JL, Casida JE (1979) Decamethrin metabolites from oxidative, hydrolytic, and conjugative reactions in mice. J Agric Food Chem 27:725-731.

Ruzo LO, Casida JE (1981) Pyrethroid photochemistry: (S)-a-cyano-3-phenoxybenzyl *cis*-(1R,3R,1'R or S)-3-(1',2'-dibromo-2',2'-dihaloethyl)-2,2-dimethylcyclopropanecarboxylates. J Agric Food Chem 29:702-706.

Saiakhov RD, Stefan LR, Klopman G (2000) Multiple computer-automated structure evaluation model of the plasma protein binding affinity of diverse drugs. Perspect Drug Discov Des 19:133-155.

Sanchez FG, Diaz AN, Pareja AG (1996) Enantiomeric resolution of pyrethroids by high-performance liquid chromatography with diode-laser polarimetric detection. J Chromatogr A 754:97-102.

Satoh T, Hosokawa M (1998) The mammalian carboxylesterases: from molecules to functions. Ann Rev Pharmacol Toxicol 38:257-288.

Satoh T, Hosokawa M (2010) Carboxylesterases: structure, function and polymorphism in mammals. J Pestic Sci 35: 218-228.

Scheuplein RJ, Blank IH (1971) Permeability of the skin. Physiol Rev 51:702-747.

Schwenkenbecker A (1904) Das absorptions verniogen der haut. Arch Anat Physiol 121-165.

Schwer H, Langmann T, Daig R, Becker A, Aslanidis C, Schmitz G (1997) Molecular cloning and characterization of a novel putative carboxylesterase, present in human intestine and liver. Biochem Biophys Res Commun 233:117-120.

Scollon EJ, Starr JM, Godin SJ, DeVitro MJ, Hughes MF (2009) In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome P450 isoforms. DMD 37:221-228.

Scott RC, Ramsay JD (1987) Comparison of the in vivo and in vitro percutaneous absorption of a lipophilic molecule (cypermethrin, a pyrethroid insecticide). J Invest Dermatol 89:142-146.

Serat WF (1973) Calculation of a safe reentry time into an orchard treated with a pesticide chemical which produces a measurable physiological response. Arch Environ Contam Toxicol 1:170-181.

Serat WF, Bailey JB (1974) Estimating the relative toxicologic potential of each pesticide in mixture of residues on foliage. Bull Environ Contam Toxicol 12:682-686.

Serat WF, Mengle DC, Anderson, HP, Kahn E (1975) On the estimation of worker entry intervals into pesticide treated fields with and without the exposure of human subjects. Bull Environ Contam Toxicol 13:506-512.

Shafer TJ, Meyer DA, Crofton KM (2005) Developmental neurotoxicity of pyrethroid insecticides: Critical review and future research needs. Environ Health Perspect 113:123-135.

Sidon EW, Moody RP, Franklin CA (1988) Percutaneous absorption of *cis*- and *trans*-permethrin in rhesus monkeys and rats: Anatomic site and interspecies variation. J Toxicol Environ Health, Part A 23:207-216.

Smith PA, Thompson MJ, Edwards JW (2002). Estimating occupational exposure to the pyrethroid termiticide bifenthrin by measuring metabolites in urine. J Chromatogr B 778:113-120.

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

Song J-H, Narahashi T (1996) Modulation of sodium channels of rat cerebellar Purkinje neurons by the pyrethroid tetramethrin. J Pharmacol Exp Ther 277:445-453.

Sorich MJ, McKinnon RA, Miners JO, Smith PA (2006) The importance of local chemical structure for chemical metabolism by human uridine 5'-diphosphate-gluronyltransferase. J Chem Inf Model 46:2692-2697.

Southwood J (1985a) PP321: Acute oral toxicity studies. Zeneca Agricultural Products Laboratory Project No. CTL/P/1102, Wilmington, DE.

Southwood J (1985b) PP993: Acute oral toxicity, acute intraperitoneal toxicity, and acute dermal toxicity studies. Zeneca Agricultural Products Laboratory Project No. CTL/P/986, Wilmington, DE.

Staiger LE, Quistad GB (1984) Metabolism of [benzyl-U-ring-^{14}C] fluvalinate by rats. J Agric Food Chem 32:1130-1133.

Stephens RH, O'Neill CA, Warhurst A, Carlson GL, Rowland M, Warhurst G (2001) Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia. J Pharmacol Exp Ther 296:584-591.

Stok JE, Huang H, Jones PD, Wheelock CE, Morisseau C, Hammock BD (2004) Identification, expression, and purification of a pyrethroid-hydrolyzing carboxylesterase from mouse liver microsomes. J Biol Chem 279: 29863-29869.

Symington SB, Frisbie RK, Clark JM (2008) Characterization of 11 commercial pyrethroids on the functional attributes of rat brain synaptosomes. Pesti Biochem Physiol 92:61-69.

Sudhahar CG, Haney RM, Xue Y, Stahelin RV (2008) Cellular membranes and lipid-binding domains as attractive targets for drug development. Curr Drug Targets 9:603-613.

Suzuki T, Miyamoto J (1974) Metabolism of tetramethrin in houseflies and rats in vitro. Pest Biochem Physiol 4:86-97.

Suzuki T, Ohno N, Miyamoto J (1976) New metabolites of (+)-*cis*-fenothrin, 3 phenoxybenzyl (+)-*cis*-chrysanthemumate, in rats. J Pestic Sci 1: 151-152.

Tamai I, Takanaga H, Maeda H, Sai Y, Ogihara T, Higashida H, Tsuji A (1995) Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acid. Biochem Biophys Res Comm 214:482-489.

Tan HL, Bezzina CR, Smits JPP, Verkerk AO, Wilde AAM (2003) Genetic control of sodium channel function. Cardiovasc Res 57:961-973.

Tan J, Liu Z, Wang R, Huang ZY, Chen AC, Gurevitz M, Dong K (2005) Identification of amino acid residues in the insect sodium channel critical for pyrethroid binding. Mol Pharmacol 67:513-522.

Tan X, Hou S, Wang M (2007) Enantioselective and diastereoselective separation of synthetic pyrethroid insecticides on a novel chiral stationary phase by high-performance liquid chromatography. Chirality 19:574-580.

Tatebayashi H, Narahashi T (1994) Differential mechanism of action of the pyrethroid tetramethrin on tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels. J Pharmacol Exp Ther 270:595-603.

Tateno C, Ito S, Tanaka M, Yoshitake A (1993) effects of pyrethroid insecticides on gap junctional intercellular communications in Balb/c3T3 cells by dye-transfer assay. Cell Biol Toxicol 9:215-221.

Timchalk C, Nolan RJ, Mendrala AL, Dittenber DA, Brzak KA, Mattsson JL (2002) A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci 66:34-53.

Tomigahara Y, Mori M, Shiba K, Isobe N, Kaneko H, Nakatsuka I, Yamada H (1994a) Metabolism of tetramethrin isomers in rat. I. Identification of a sulfonic acid type of conjugate and reduced metabolites. Xenobiotica 24:473-484.

Tomigahara Y, Mori M, Shiba K, Isobe N, Kaneko H, Nakatsuka I, Yamada H (1994b) Metabolism of tetramethrin isomers in rat. II. Identification and quantification of metabolites. Xenobiotica 24:1205-1214.

Tomigahara Y, Shiba K, Isobe N, Kaneko H, Nakatsuke I, Yamada H (1994c) Identification of two new types of S-linked conjugates of Etoc in rat. Xenobiotica 24:839-852.

Tomigahara Y, Onogi M, Miki M, Yanagi K, Shiba K, Kaneko H, Nakatsuka I, Yamada H (1996) Metabolism of tetramethrin isomers in rat. III. Stereochemistry of reduced metabolites. Xenobiotica 26:201-210.

Tomigahara Y, Onogi M, Saito K, Kaneko H, Nakatsuka I, Yamane S (1997) Metabolism of tetramethrin in rat. IV. Tissues responsible for formation of reduced and hydrated metabolites. Xenobiotica 27(9):961-971.

Tornero-Velez R, Mirfazaelian A, Kim KB, Anand SS, Kim HJ, Haines WT, Bruckner JV, Fisher JW (2010) Evaluation of deltamethrin kinetics and dosimetry in the maturing rat using PBPK model. Toxicol Appl Pharmacol 244: 208-217.

Tsuji T, Kaneda N, Kado K, Yokokura T, Yoshimoto T, Tsuru D (1991) CPT-11 converting enzyme from rat serum: purification and some properties. J Pharmacobiodyn 14:341-349.

Tubic M, Wagner D, Spahn-Langguth H, Bolger MB, Langguth P (2006) In silico modeling of non-linear drug absorption for the P-gp substrate Talinolol and of consequences for the resulting pharmacodynamic effect. Pharmaceut Res 23:1712-1720.

Tullman RH (1987) Data Evaluation Report No. 005731, U.S. Environmental Protection Agency, Washington, DC.

Ueda K, Gaughan LC, Casida, JE (1975) Metabolism of (+)-*trans*- and (+)-*cis* -Resmethrin in rats. J Agr Food Chem 23:106-115.

Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V, Riordan JR (1986) The mdr1 gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem Biophys Res Commun 141:956-962.

Ulrich E, Sey YM, Harrison RA, DeVito MJ (2008) LC/MS method for the analysis of permethrin enantiomers in biological matrices. 20th International Symposium on Chirality, Geneva, Switzerland, July 6-9, 2008.

Unai T, Casida JE (1977) Synthesis of isomeric 3-(2,2-Dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic acids and other permethrin metabolites. J Agric Food Chem 25:979-987.

Valverde A, Aguilera A, Rodriguez M, Boulaid M (2001) What are we determining using gas chromatographic multiresidue methods: tralomethrin or deltamethrin? J Chromatogr A, 943(1):101-111.

Van der Rhee HJ, Farquhar JA, Vermeulen NP (1989) Efficacy and Transdermal absorption of permethrin in scabies patients. Acta Dermatol Venerol Stockh 69:170-173.

Varsho, BJ (1996) Acute oral toxicity study of deltamethrin in albino rats. AgrEvo USA Company Study No. WIL-274001.

Venkatakrishnan K, Von Moltke LL, Obach RS, Greenblatt DJ (2000) Microsomal binding of amitriptyline: effect on estimation of enzyme kinetic parameters in vitro. J Pharmacol Exp Ther 293:343-350.

Vistoli G, Pedretti A, Mazzolari A, Testa B (2010) In silico prediction of human carboxylesterase-1 (hCES1) metabolism combining docking analysis and MD simulations. Bioorg Med Chem 18:320-329.

Vozeh S, Taeschner W, Wenk M (1990) Pharmacokinetic drug data. In: Clinical pharmacokinetics drug data handbook. Auckland, NZ: ADIS Press, pp 1-38.

Wadkins RM, Hyatt JL, Wei X, Yoon KJP, Wierdl M, Edwards CC, Morton CL, Obenauer JC, Damodaran K, Beroza P, Danks MK, Potter PM (2005) Identification and characterization of novel benzyl (diphenylethane-1,2-dione) analogues as inhibitors of mammalian carboxylesterases. J Med Chem 48:2906-2915.

Wang P, Zhou Z, Jiang S, Yang L (2004) Chiral resolution of cypermethrin on cellulose-tris(3,5-dimethylphenyl-carbamate) chiral stationary phase. Chromatographia 59:625-629.

Wang Q, Qiu J, Zhu W, Jia G, Li J, Bi C, Zhou Z (2006) Stereoselective degradation kinetics of theta-cypermethrin in rats. Environ Sci Technol 40:721-726.

Wang M, Wang Q, Hong C, Sleczka B, D'Arienzo C, Josephs J, Ye X-Y, Robl J, Gordon D, Rodrigues D, Harper T (2010) Prediction of in vivo enantiomeric compositions by modeling in vitro metabolic profiles. J Pharma Sci 99:3234-3245.

Waxman SG, Craner MJ, Black JA (2004) Na+ channel expression along axons in multiple sclerosis and its models. Trends Pharmacol Sci 25:584-591.

Weiner ML, Nemec M, Sheets L, Sargent D, Breckenridge (2009) Comparative functional observational battery study of twelve commercial pyrethroid insecticides in male rate following oral exposure. Neurotoxicology 30S:S1-S16.

Wester RC, Maibach HI (1977) In: Drill VA, Lazar P (eds.), Cutaneous Toxicology, Academic Press, New York, p 111

Wester RC, Noonan PK (1980) Relevance of animal-moles for percutaneous-absorption. Int J Pharm 7:99-110.

Wester RC, Maibach HI (1993) Animal models for percutaneous absorption. In: Wang RGM, Knaak JB, Maibach HI (eds.), Health Risk Assessment: Dermal and Inhalation Exposure and Absorption of Toxicants. CRC Press, Boca Raton, FL.

Wester RC, Bucks DAW, Maibach HI (1994) Human in vivo percutaneous absorption of pyrethrin and piperonyl butoxide. Food Chem Toxicol 32:51-53.

Wheelock CE, Wheelock AM, Zhang R, Stok JE, Morisseau C, Le Valley SE (2003) Evaluation of alpha-cyanoesters as fluorescent substrates for examining interindividual variation in general and pyrethroid-selective esterases in human liver microsomes. Anal Biochem 315:208-222.

Wheelock CR, Miller JL, Miller MJ, Phillips BM, Huntley SA, Gee SJ, Tjeerdem RS, Hammock BD (2006) Use of carboxylesterase activity to remove pyrethroid-associated toxicity to Ceriodaphnia dubia and Hyalella azteca toxicity in identification evaluations. Environ Toxicol Chem 25:973-984.

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

White S (1994) Protein Membrane Structure. Oxford University Press, Oxford.

WHO (World Health Organization) (1965) Evaluation of the toxicity of pesticide residues in food: dimethrin. FAO meeting report PL/1965/10/1. Food and Agriculture Organization (United Nations), www.inchem.org/documents/jmpr/jmpmono/v065pr21.htm, 1965.

WHO (2005a)WHO Specifications and evaluations for public health pesticides, Deltamethrin, (S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate. WHO, Genève.

WHO (2005b) The WHO Recommended Classification of Pesticides by Hazard. Guidelines to Classification 2004, International Programme on Chemical Safety. WHO/PCS/01.5, Genève, , http://www.who.int/ipcs/publications/pesticides_hazard/en/.

Williams RL, Bernard, CE, Krieger RI (2003) Human exposure to indoor residential cyfluthrin residues during a structured activity program. J Exp Anal Environ Epidemiol 13:112-119.

Wilson ZE, Rostami-Hodjegan A, Burn JL, Tooley A, Boyle J, Ellis SW and Tucker GT (2003) Inter-individual variability in levels of human microsomal protein and hepatocellularity per gram of liver. Br J Clin Pharmacol 56**:**433-440.

Wolansky MJ, Harrill JA (2008) Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. Neurotoxicol Teratol 30:55-78.

Woollen BH, Marsh JR, Laird, WJD, Lesser JE (1992) The metabolism of cypermethrin in man: differences in urinary profiles following oral and dermal administration. Xenobiotica 22:983-991.

Wu A, Liu Y (2003) Prolonged expression of c-Fos and c-Jun in the cerebral cortex of rats after deltamethrin treatment. Brain Res Mol Brain Res 110:147-151.

Xu C, Wang J, Liu W, Sheng GD, Tu Y, Ma Y (2007) Separation and aquatic toxicity of enantiomers of the pyrethroid insecticide Lambda-Cyhalothrin. Environ Toxicol Chem 27:174-181.

Yamamoto I, Elliot M, Casida JE (1971) Metabolic fate of pyrethrin I, pyrethrin II, and allethrin. Bull World Health Organ 44:347-348.

Yamazaki K, Kanaoka M (2004) Computational prediction of the plasma protein-binding percent of diverse pharmaceutical compounds. J Pharm Sci 93:1480-1494.

Yang J, Tucker GT, Rostami-Hodjegan A (2004) Cytochrome P450 3A expression and activity in the human small intestine. Clin Pharmacol Ther 76:931.

Yang G-S, Vazquez PP, Frenich AG, Vidal JLM, Aboul-Enein HY (2004) Separation on CHIRALCEL OD-R with methanol/water, and acetonitrile/water mobile phases. J Liq Chromatogr Rel Technol 27(10):1507-1521.

Yoon KJ-P, Krull EJ, Morton CL, Bornmann WG, Lee RE, Potter PM, Danks MK (2003) Activation of a camptothecin prodrug by specific carboxylesterases as predicted by quantitative structure-activity relationship and molecular docking studies. Mol Cancer Ther 2:1171-1181.

Youdim KA, Zayed A, Dickins M, Phipps A, Griffiths M, Darekar A, Hyland R, Fahmi O, Hurst S, Plowchalk DR, Cook J, Guo F, Obach RS (2008) Application of CYP3a4 in vitro data to predict clinical drug-drug interactions; predictions of compounds as objects of interaction. Br J of Clin Pharmacol 65:680-692.

Youdim K, Dodia R (2010) Comparison between recombinant P450s and human liver microsomes in the determination of cytochrome P450 Michaelis-Menten constants. Xenobiotica 1-10, Early Online.

Yu LX, Lipka E, Crison JR, Amidon GL (1996) Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. Adv Drug Del Rev 19:359-376.

Zeng S, Tang Y (2009) Effect of clustered ion channels along an unmyelinated axon. Physical Review E 80:1-9.

Zhang H (2005) A new approach for the tissue-blood partition coefficients of neutral and ionized compounds. J Chem Inform Model 45:121-127.

Zhang X, Tsang AM, Okino MS, Power FW, Knaak JB, Harrison LS, Dary CC (2007) A physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model for carbofuran in Sprague-Dawley rats using the Exposure Related Dose Estimating Model (ERDEM). Toxicol Sci 100:345-359.

Zhe X, Wenwei X, Hua H, Lirui P, Xu X (2008) Direct chiral resolution and its application to the determination of the pesticide tetramethrin in soil by high-performance liquid chromatography using polysaccharide-type chiral stationary phase. J Chromatogr Sci 46:783-786.