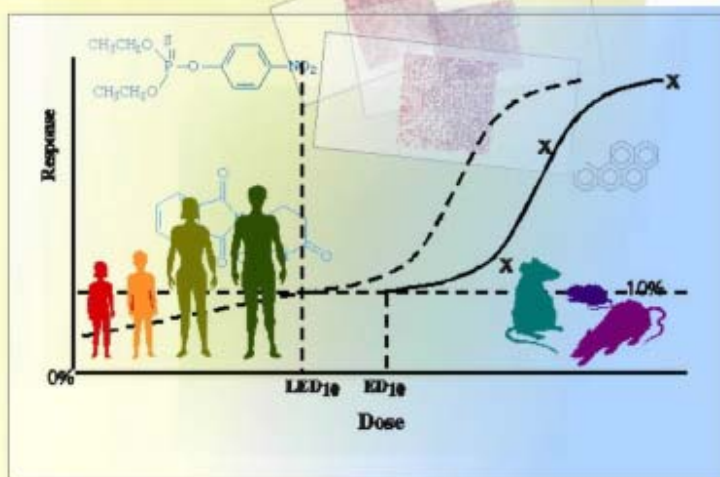


Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides

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1. Background

EPA must quantify the potential human risk that may be associated with exposure to environmental contaminants either by establishing national standards for clean air and water, regulating pesticide products by approval of registration, or setting clean-up standards for hazardous site remediation. These efforts necessitate dose-response assessment (i.e., how the frequency of adverse effects changes with decreasing dose) and typically involve extrapolations from high to low doses and from a nonhuman species to human beings. EPA has historically applied default approaches in estimating the potential human risk associated with environmental exposures; this is the case for many pesticide chemicals, including pyrethroids. Recent efforts to improve the scientific basis for risk assessments have involved consideration of chemical specific adjustment factors (often called data derived uncertainty factors; IPSC, 2005) and/or the development of sophisticated models, like physiologically based pharmacokinetic models (PBPK) or biologically based dose response models (BBDR). In recent years, the Office of Pesticide Programs (OPP) has worked collaboratively with researchers from EPA's Office of Research and Development (ORD) to improve the scientific support for risk assessments. These efforts have led to the development of cumulative risk assessments for the organophosphate and N-methyl carbamate pesticides along with mode of action analyses for dimethylarsinic acid (DMA) and atrazine. In addition, OPP and ORD have worked collaboratively on efforts to develop PBPK models for several pesticide chemicals, including carbaryl, malathion, and DMA. At present time, OPP and ORD are working together to develop PBPK models for several pyrethroid pesticides.

PBPK models are mathematical descriptions of the biological, physiological, and physiochemical properties regulating the pharmacokinetics of chemicals. While more data intensive than classical pharmacokinetic models, PBPK models offer the advantage of more quantitative extrapolations between species and exposure scenarios. At previous meetings of the FIFRA Scientific Advisory Panel (USEPA, 2004, 2005), the Agency requested comment on a variety of topics related to the development of PBPK models, particularly for the N-methyl carbamates. EPA is developing an approach to develop and apply pharmacokinetic models for several pyrethroids for potential use in dose and species extrapolation. The purpose of the present meeting of the FIFRA Scientific Advisory Panel (SAP) is to consider several scientific issues encountered by EPA in the development of these models. The Agency's PBPK models for deltamethrin and permethrin are still under development and at a stage where feedback from the SAP on the noted issues will be helpful prior to continuing. The current document and related charge questions to the panel are designed to focus on these areas where the Agency is soliciting comment. Section 2 summarizes key aspects of pyrethroid toxicology and pharmacokinetics. Section 3 describes EPA's efforts to build PBPK models for the pyrethroids and focuses on the areas where the panel is asked to provide comment. Appendices A-C contain technical details and results of key studies discussed through out this paper. Specific details about the PBPK models can be found in Appendix D. The Agency is soliciting comment on the following science issues:

- ❑ **Concept of using a common-model structure for the pyrethroid pesticides.** Pyrethroids share a common chemical structure and toxicity profile; therefore a common PBPK model for the pyrethroids with chemical specific metabolic and partitioning parameters may be of value. See sections 2.C and 2.D for evidence of common pharmacokinetic, metabolism and toxicity profiles. See Section 3 for common PBPK model structure.
- ❑ **Appropriate dose metric for the pyrethroid pesticides.** Although uncertainties surrounding aspects of the mechanism of action for pyrethroids still exist, much is known about the toxicity profile for these chemicals. Initial assessment of pyrethroid pesticides indicates that blood and/or brain concentrations may be useful dose metrics for estimating potential neurotoxic responses to pyrethroids. Section 2.F describes scientific considerations surrounding potential dose metrics.
- ❑ **Approaches to incorporate stereoisomers for pyrethroids.** In the case of pyrethroid pesticides, one of the first challenges is to identify the toxicophore. Pyrethroids have one or more chiral centers resulting in several stereoisomers. There is limited information on the toxicity and pharmacokinetics of the different stereoisomers. The Agency is proposing to evaluate several modeling assumptions including, 1) “lumping” all enantiomers and diastereomers of a pyrethroid as one chemical (e.g., a single model); 2) “lumping” all enantiomers within a diastereomer (e.g., separate models for *cis*- and *trans*-; 3) ignoring all “non-toxic” stereoisomers and model the most toxic stereoisomers (e.g., *cis*-permethrin). To evaluate these approaches, the Agency is using permethrin as a model chemical. (see section 3.C.3).
- ❑ **Species extrapolation given limited human information.** The Agency anticipates that human information surrounding pyrethroid metabolism will be limited to *in vitro* studies, metabolism and/or absorption studies in human subjects with small sample sizes and few measured parameters, and biomonitoring studies where exposure is not controlled and thus not known. A strategy for handling these data is proposed in section 3.D.

2. Pyrethroid Toxicology: Pharmacodynamic & Pharmacokinetic Profile

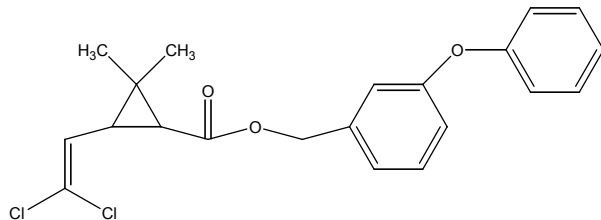
2.A. Introduction

Pyrethroids are synthetic pesticides with a chemical structure based on pyrethrins. Pyrethrins are a group of natural pesticides isolated from the plant extract pyrethrum. Pyrethrum is derived from the flowers of *Chrysanthemum cinerariaefolium* and *Chrysanthemum cineum*. The basic pyrethrin structure was altered such that the developed products, the pyrethroids, have enhanced photostability, insecticidal activity, and also mammalian toxicity. Pyrethroids are used in agricultural and residential settings to control a variety of insects. Individuals can be exposed to pyrethroids via inhalation, oral ingestion, or dermal contact. The following section summarizes key information about the chemical structure, toxicity profiles, and pharmacokinetic properties for these pesticides. This is not meant to be a thorough review of pyrethroid toxicology. For more detailed reviews of pyrethroid toxicology and metabolism see Soderlund et al. (2002), Shafer and Meyer (2004), Shafer et al. (2005), and Ray and Fry (2006).

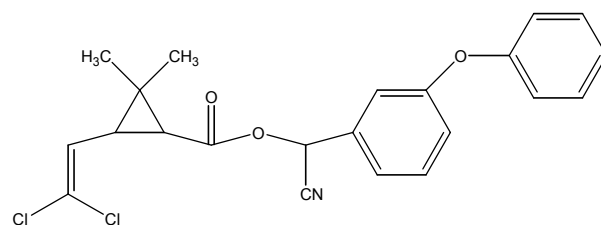
2.B. Chemical Structure

Pyrethroids are composed of two basic structural moieties, an acid and an alcohol. For first generation pyrethroids, the acid portion is based on chrysanthemic acid, a cyclopropane ring bonded to a carboxylic acid moiety and a variety of halogenated and non-halogenated substituents. Later developed pyrethroids, such as fenvalerate, do not have a cyclopropane ring. The alcohol portion is either a primary or a secondary alcohol, which is bound to a variety of heterocyclic structures. In addition, several of the pyrethroids have a cyano substituent bound to the α -methylene of the alcohol, which results in enhanced toxicity of the compound. Pyrethroids lacking the α -cyano substituent are termed Type I compounds and the pyrethroids with the α -cyano substituent are termed Type II compounds. Figure 2.1 shows the structures of a Type I pyrethroid, permethrin, and a Type II pyrethroid, cypermethrin.

Figure 2.1 The structures of permethrin and cypermethrin



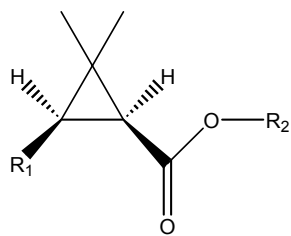
Permethrin



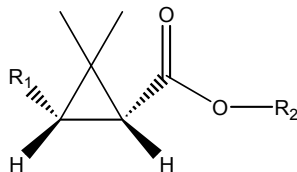
Cypermethrin

An important aspect of the chemical and toxicological properties of pyrethroids is their overall configuration. A pyrethroid's configuration influences its toxic potency and predominant pathway for metabolism. The *cis* and *trans* designation indicates how a substituent on carbon-3 of the cyclopropane ring is oriented in relation to the carboxylic acid group bound to carbon-1 (Figure 2.2). *Cis* implies the carboxylic acid on carbon-1 and the substituent on carbon-3 are oriented on the same side, whereas *trans* implies they are on opposite sides. Pyrethroids can have 1-4 chiral centers. Thus, pyrethroids with 3 chiral centers, such as cypermethrin, have 8 stereoisomers. Some commercial formulations are sold as several isomers of one pyrethroid, whereas deltamethrin is marketed as a single stereoisomer (purity $\geq 98\%$) (Figure.2.3).

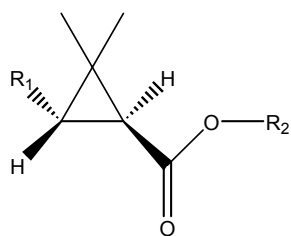
Figure 2.2 Stereochemical configurations of the chrysanthemic acid moieties. Carbon-1 is bound to the carboxylic acid moiety and carbon-3 is bound to the R₁ moiety



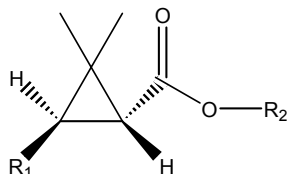
1S, 3R cis



1R, 3S cis

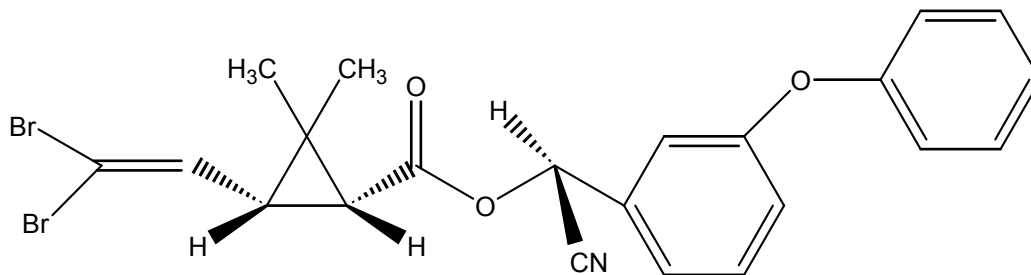


1S, 3S trans



1R, 3R trans

Figure 2.3 Structure of deltamethrin



There are differences in the metabolism and toxicological effects of the pyrethroid stereoisomers. The most toxicologically active compounds in mammals have the structure of 1R *cis* (cyano α S) (e.g., deltamethrin) followed by the 1R *trans* (cyano α S) configuration. Those that have the structure of 1S *cis* (cyano α R) or 1S *trans* (cyano α R) are the least active. Table 2.1 demonstrates this pattern with the toxicity of permethrin isomers in mice.

Table 2.1 Acute oral toxicity of permethrin isomers in mice^a

Compound	Male	Female
Racemic Permethrin	490 ^b	490
1R- <i>cis</i> permethrin	107	85
1R- <i>trans</i> permethrin	3100	3200
1S- <i>cis</i> permethrin	>5000	>5000
1S- <i>trans</i> permethrin	>5000	>5000

^aData from Miyamoto (1976)

^bLD₅₀, mg/kg

Pyrethroids with the *trans* configuration and a primary alcohol (e.g., *trans*-permethrin) are hydrolyzed more readily by esterases than those with the *cis* configuration (e.g., *cis*-permethrin). This explains in part the reason pyrethroids with the *trans* configuration demonstrate less mammalian toxicity than those with the *cis* configuration.

For some pyrethroids with multiple enantiomers a variety of commercial products are available. Typically Greek letters, and bio- and es- prefixes indicate these compositional changes. For example, *alpha*-, *beta*-, *theta*-, and *zeta*-cypermethrin all refer to the same chemical, but each have a different percentage of the eight possible enantiomers (Tomlin, 2000). Table 2.2 shows five different formulations of allethrin, each having a distinctive pattern of enantiomer composition (Williams, 1992).

Table 2.2 Enantiomer composition of allethrin formulations

Enantiomer	Allethrin	Allethrin Forte	Bioallethrin	Esbiothrin	Esbiol
1R <i>trans</i> S (Most insecticidal)	18	36.8	46.5	72	90
1R <i>trans</i> R	18	36.8	46.5	21	5
1R <i>cis</i> S	18				
1R <i>cis</i> R	18				
1S <i>cis</i> S	4.5	9.2			
1S <i>cis</i> R	4.5	9.2			
1S <i>trans</i> R	4.5				
1S <i>trans</i> S (least insecticidal)	4.5				

In addition to causing nomenclature confusion, these changes in enantiomer composition also have an impact on the insecticidal properties, exposures, and toxicities of the formulation. One effect of these compositional changes is that more recent products, such as Esbiol (*S*-bioallethrin), have a higher percentage of the more efficacious enantiomers (1*R trans* *S*), and very little of the ineffective ones (1*S trans* *S*). This means that the application rate of the pesticide can be decreased but retain the same efficacy. These newer more effective product with a different enantiomer profile from the original product result in less exposure to humans and non-target organisms (Williams, 1992).

Although pyrethroid toxicity varies with enantiomer composition, physical mechanisms such as UV degradation, volatilization, and OH radical reactions generally do not vary with enantiomer composition. Some pyrethroids undergo photolysis reactions or isomerization that can change the enantiomer profile under specific conditions (Maguire, 1990; Persche and Hussain, 1992). Biologically mediated degradation pathways frequently change enantiomer composition due to the presence of a chiral environment. For example, in a soil media, 1*R-cis*-permethrin degrades more slowly than 1*S-cis*-permethrin under aerobic conditions (Qin and Gan, 2006). In general, the *trans*-isomers of pyrethroids degrade faster in the environment than the *cis*-isomers (Liu and Gan, 2004; Qin et al., 2006). Enantioselective degradation of contaminants adds another level of difficulty in assessing exposure because the applied mixture may be altered before the exposure.

After an organism has been exposed, additional enantioselective reactions may take place. Enantioselective behavior is often observed for metabolism, uptake, excretion, and transport *in vivo* for some other environmental contaminants (Williams, 1992). The (+) enantiomer of α -hexachlorocyclohexane is preferentially found in the brain of several species, and researchers have hypothesized this is due to enantioselective transport across the blood-brain barrier (Mossner et al., 1992; Huhnerfuss et al., 1993; Moller et al., 1993; Vetter and Schurig, 1997; Iwata et al., 1998; Ulrich et al., 2001). Enantioselective degradation of polychlorinated biphenyl (PCB) atropisomers has been found in fish, organisms not previously thought to have much capacity to biotransform PCBs (Wong et al., 2002, 2004). While the *in vitro* stereoselective metabolism of pyrethroids has been examined, there is little information on the influence of chiral chemistry on the overall pharmacokinetics of pyrethroids. The lack of readily available purified enantiomers for pyrethroids has limited the development of data sets that can examine the toxicity and pharmacokinetics of these chemicals.

One difficulty in the incorporation of chiral chemistry into a risk assessment for pyrethroids is the level of chiral chemistry resolution of the available data. Much of the toxicity data examines the technical products prior to formulation. As described above, these products are typically mixtures of stereoisomers of a single pyrethroid. Exposure information on the stereoisomer composition is available at the diastereomers and/or enantiomer level depending upon the specific chemical and formulation. For example, presently, monitoring data, such as the USDA's Pesticide Data Program (PDP), does not routinely resolve pyrethroids at the enantiomer level (Table 2.3). PDP does report several pyrethroids, such as permethrin, at the diastereomer level. In a national survey

of pesticide residues in child care centers (Tulve et al, 2006) levels of *cis*- and *trans*-permethrin were reported, but *cis*- and *trans*-cypermethrin were not differentiated.

Table 2.3 Resolution of pyrethroid residues reported in the 2005 Annual Summary of the Pesticide Data Program, U.S. Department of Agriculture

Pyrethroid Form Reported In PDP database	Actual # of diastereomers of agricultural product	Actual # of enantiomers of agricultural product
Allethrin	4	8
Bifenthrin	1	2
Cyfluthrin	4	8
Cyhalothrin, total	4	8
λ -cyhalothrin	1	2
Cyhalothrin, R-157836	1	2
Cypermethrin	4	8
Deltamethrin	1	1
Esfenvalerate	2	4
Fenpropathrin	1	2
Fenvalerate	2	4
Permethrin, total	2	4
<i>cis</i> -Permethrin	1	2
<i>trans</i> -Permethrin	1	2
Phenothrin	2	2
Prallethrin	4	4
Resmethrin, total	2	4
<i>cis</i> -Resmethrin	1	2
<i>trans</i> -Resmethrin	1	2
Tetramethrin	2	4
Tefluthrin	1	2

The chiral chemistry resolution of the available data can have significant impact on PBPK model structure and uncertainty. Furthermore, the approaches and assumptions used to address issues related to chiral chemistry can have significant impacts on the risk assessment of this class of chemicals. Because of the difficulties in evaluating the chiral chemistry of pyrethroids, the Agency's first efforts to build a PBPK model for pyrethroids focused on deltamethrin, which is one enantiomer. The Agency is using permethrin, which consists of two diastereoisomers (*cis* and *trans*) and four enantiomers, as a case study for ways to handle more complicated situations regarding the chiral chemistry of pyrethroids.

2.C. Toxicity

Pyrethroids are neurotoxic agents which are typically categorized as either Type I or Type II compounds. As described in the previous section, Type II, but not Type I compounds, contain a cyano substituent on the α -methylene of the alcohol moiety. The neurotoxic behaviors elicited by Type I pyrethroids in laboratory animals are aggression, hyperexcitability, fine tremor, prostration with coarse whole body tremor, increased body temperature, coma and death. These neurobehavioral responses are termed the T-syndrome because of the fine tremors induced by the Type I pyrethroids. For Type II

pyrethroids, the neurotoxic behaviors include pawing and burrowing, salivation, hyperexcitability, abnormal hind limb movements, coarse whole body tremor, sinuous writing or choreoathotosis, coma and death. These neurobehavioral responses are termed the CS-syndrome for the choreoathotosis and salivation observed in laboratory animals. Some pyrethroids such as fenpropathrin and cyphenothrin may elicit mixed behaviors of the T- and CS-syndromes. The T- and CS-syndromes are considered to be acute responses to exposure to pyrethroids and are dose-dependent. Dermal exposure may result in parasthesia, a tingling or burning sensation of the directly contacted skin and is thought to result from hyperactivity of cutaneous sensory nerve fibers.

While there is a substantial body of evidence suggesting a prominent role of the sodium channels in the toxicity of Type I and II pyrethroids, uncertainties remain surrounding their mechanism(s) of toxicity particularly since there is also evidence that alterations of chloride, calcium and other channels by pyrethroids may also play a role in the toxicity of these chemicals. Moreover, the pyrethroid sodium channel binding site has not been identified. Type I and II pyrethroids are believed to slow the activation (opening) and the inactivation (closing) of the sodium channels. These delays in opening and closing of the sodium channel prolong the sodium current. The length of this current is dependent on whether the chemical is a Type I or Type II pyrethroid. The Type I pyrethroids open the channel just long enough to cause a repetitive firing of the neuron. The Type II pyrethroids hold the channel open long enough so that the neuron becomes depolarized and no longer fires (Soderlund et al., 2002; Ray and Fry, 2006).

Research efforts by both the EPA and the Pyrethroid Working Group (PWG; a group of pesticide companies which register pyrethroids) are aimed at better understanding the mechanism of toxicity for pyrethroids. EPA's ORD is performing research on the neurotoxic potential of pyrethroids by evaluating the dose-response of motor activity, changes in body temperature, and auditory startle in rats in addition to *in vitro* studies using intact neurons. The PWG recently submitted to EPA a series of specially designed functional operational battery (FOB) studies with rats to evaluate behavioral effects specific to Type I and II pyrethroids along with *in vitro* studies designed to evaluate effects on the sodium, calcium, and chloride channels. The Agency's review of the PWG studies has only recently begun and is in its early stages. In the future, the Agency may bring issues related to mechanism or mode of action for the pyrethroids to the FIFRA SAP for review.

2.D. Pharmacokinetics

2.D.1. Oral Absorption

Absorption of pyrethroids administered orally to rats occurs rapidly with blood concentrations reaching maximal levels at 2-4 hr post-administration. Absorption of pyrethroids does not appear to be complete. Up to 14% of administered deltamethrin (Ruzo et al., 1978) and 20% of cypermethrin (Crawford et al., 1981b) have been identified intact in feces of orally treated rats. In addition, the oral bioavailability of deltamethrin is low (14%), whereas it is higher for permethrin and λ -cyhalothrin (both about 60%). The mechanism of the absorption of pyrethroids is thought to be by simple diffusion. In order to account

for the incomplete absorption of pyrethroids, it has been suggested that efflux transporters along the intestinal tract limit the absorption of these chemicals (Mirfazaelian et al. 2006). If efflux transporters are involved in the incomplete absorption of pyrethroids, there should be greater absorption of these chemicals with increasing dose due to saturation of the transporters. However, limited and inconsistent data on the potential dose dependent oral absorption and bioavailability of pyrethroids are available to determine whether the incomplete absorption is due to efflux transporters or due to the limited solubility of high concentrations of pyrethroids. Ongoing efforts at ORD are experimentally evaluating this issue.

Some factors that influence oral absorption of pyrethroids are vehicle, dosing volume, and route of administration/exposure. Crofton et al. (1995) examined the effect of vehicle and route of administration of deltamethrin on motor activity in rats (Table 2.4). Vehicles included corn oil, glycerol formal, Emulphor®, and methylcellulose. The dosing volume was 1 ml/kg for corn oil, glycerol formal, and Emulphor® and 3 ml/kg for methylcellulose. They observed a 200-fold difference in the effective dose that produced a 50% decrease (ED₅₀) in motor function of the animals when comparing vehicles and oral administration and >20-fold difference when comparing vehicles and intraperitoneal (ip) administration. When comparing routes within a vehicle, disparate results were observed. For corn oil, the oral route resulted in greater toxicity than the ip route. For glycerol formal, the routes of administration did not influence the ED₅₀. For Emulphor® and methylcellulose, the ip route resulted in a greater ED₅₀ than following oral administration.

Table 2.4: ED₅₀ values (mg/kg) for motor activity the effect of deltamethrin on motor function in rats

Vehicle	Oral	Intraperitoneal
Corn Oil	5.1	38.9
Emulphor®	>200.0	7.1
Glycerol formal	8.1	7.6
Methylcellulose	>1000	160.1

Dosing volume can also affect the outcome of motor activity in rats exposed to pyrethroids. Wolansky et al. (2007) recently examined the effect of bifenthrin administered in corn oil with volumes of either 1 or 5 ml/kg. They reported that the effects of bifenthrin on motor activity and pyrethroid-specific clinical signs were approximately 2-fold more potent at 1 ml/kg than at 5 ml/kg.

These findings are significant because of the lack of conformity among laboratories in the experimental conditions used to study the neurotoxicological effects of pyrethroids. As the Agency moves forward in evaluating the dose response relationship of pyrethroids and in comparing the toxic potency of pyrethroids across different toxicity studies, impact of administration vehicle and volume may need to be taken into account.

2.D.2. Dermal Absorption

Dermal exposure to pyrethroids can occur in occupational or non-occupational settings. For dermal absorption to occur, a pyrethroid would first contact the stratum corneum, the outermost layer of skin. The stratum corneum is part of the epidermis, but is not a viable tissue. It consists primarily of the protein keratin and a complex mixture of lipids. For most chemicals, the main barrier for penetration is the stratum corneum. Below the stratum corneum are the viable epidermis and dermis, the latter being vascularized, and more of an aqueous area than the upper layers of the epidermis. Chemicals that diffuse into the dermis are available to reach the systemic circulation.

Dermal absorption is a process of passive diffusion. Thus there should be no difference in the dermal absorption of pyrethroid stereoisomers, as observed with *cis*- and *trans*-permethrin in the rat (Sidon et al., 1988). Penetrating the stratum corneum is the rate limiting step for dermal absorption of most chemicals. Several parameters can influence dermal absorption of a chemical. These include the physical and chemical properties of the compound, the vehicle and the biological properties of the skin. Regarding the chemical, molecular weight (or size), polarity (ionization and hydrogen bonding potential), and lipid solubility are important parameters. As a generality, pyrethroids have a low ionization and hydrogen bonding potential, molecular weight in the 300 – 400 range and a high lipid solubility. A measurement of a chemical's lipid solubility is the log octanol:water partition coefficient or log P. Chemicals with a log P of 1-2 readily dissolve in organic and aqueous solvents and are generally well absorbed through skin. Pyrethroids have a high log P (ca. 6). Because these chemical are more lipid soluble than water soluble, they may not penetrate completely through the skin. Biological parameters that can influence dermal absorption include species, site of exposure, metabolism, age and skin circulation, hydration, integrity, and temperature.

The dermal absorption data of pyrethroids in the literature generally follows what is known about many other chemicals. Mouse skin tends to be more permeable to chemicals than rat skin, which is slightly more permeable than pig and human skin. Absorption of permethrin through mouse skin is greater than in rat and monkey skin (Shah et al., 1981; Sidon et al., 1988). Cypermethrin is more permeable through rat than human skin (Scott and Ramsey 1987; Eadsforth et al., 1988; Woollen et al., 1992).

Studies by EPA have focused on the *in vitro* dermal absorption of radiolabeled bifenthrin, deltamethrin, and *cis*-permethrin in dermatomed male rat and human cadaver skin (Appendix A). Fifty percent or more of the applied dose of pyrethroid could be removed by a soap/water wash from rat and human skin 24 hr post-application of chemical. Of the radioactivity that remained in the rat skin, approximately 20% was removed by tape stripping the skin. This indicates that this portion of the dose diffused into the upper layers of the stratum corneum and could not be washed off. For both types of skin, 5% or less of the applied dose of the three pyrethroids completely penetrated the skin in 24 hr. An important determinant in the dermal absorption of pyrethroids may be the rate of absorption. Because there are esterases in rat serum that hydrolyze pyrethroids, the compound that penetrates the skin would be expected to be cleared rapidly. However, human serum does not appear to have pyrethroid hydrolyzing activity (Godin et al., 2007), so in order for humans to metabolize the dermally absorbed pyrethroid, it would need to go to an organ that could metabolize it (e.g., liver).

2.D.3. Pulmonary Absorption

The literature on the pharmacokinetics of inhalation exposure to pyrethroids in laboratory animals and humans is limited. Rats exposed to aerosols of deltamethrin showed the same clinical symptoms following administration via oral and intravenous routes (Kavlock et al., 1979).

2.D.4. Distribution

Once absorbed, the pyrethroids distribute rapidly throughout the animal. Gray and Rickard (1981) administered deltamethrin intravenously to rats at one dose level. The deltamethrin was ¹⁴C-labeled in either the acid, alcohol or cyano moieties. Within one minute of administration of any of the three radiolabeled deltamethrin compounds, radioactivity was detected in all tissues examined. Peak levels of radioactivity in brain were detected within 1-5 min. All of the animals progressively displayed the signs of Type II neurotoxicity following administration of deltamethrin. Salivation was observed from 2 to 16 min, whole body tremor 5-10 min, and choreoathetosis from 20 min post-administration. The tissue distribution time-course curves for the three radiolabeled preparations of deltamethrin were similar over 8 hr. Radioactivity remained highest in fat over this time. There was a progressive accumulation of radioactivity derived from the cyano-labeled deltamethrin in erythrocytes, which is likely due to the cleavage of the cyano group and binding of it to methemoglobin.

Residues of pyrethroids in fat tend to be higher than other tissues and these levels persist longer. The *cis* isomer of cypermethrin (¹⁴C-alcohol moiety) in fat has a half-life of about 12 days compared to less than 1 day in other tissues (Crawford et al., 1981a). The *cis* isomer of cypermethrin also persists longer than the *trans* isomer in fat (Crawford et al., 1981a). After repeated oral dosing of cypermethrin, the highest concentration of radioactivity was in the fat (Rhodes et al., 1984).

It is not known how pyrethroids distribute throughout the blood. In the study by Gray and Rickard (1981), the plasma contained the major portion of the radioactivity derived from the alcohol and acid-labeled moieties of deltamethrin. Increased radioactivity was detected in the erythrocytes over time following the administration of cyan-labeled deltamethrin. While information on serum binding is limited, permethrin does not interact with human serum albumin (Abu-Qare and Abou-Donia, 2002).

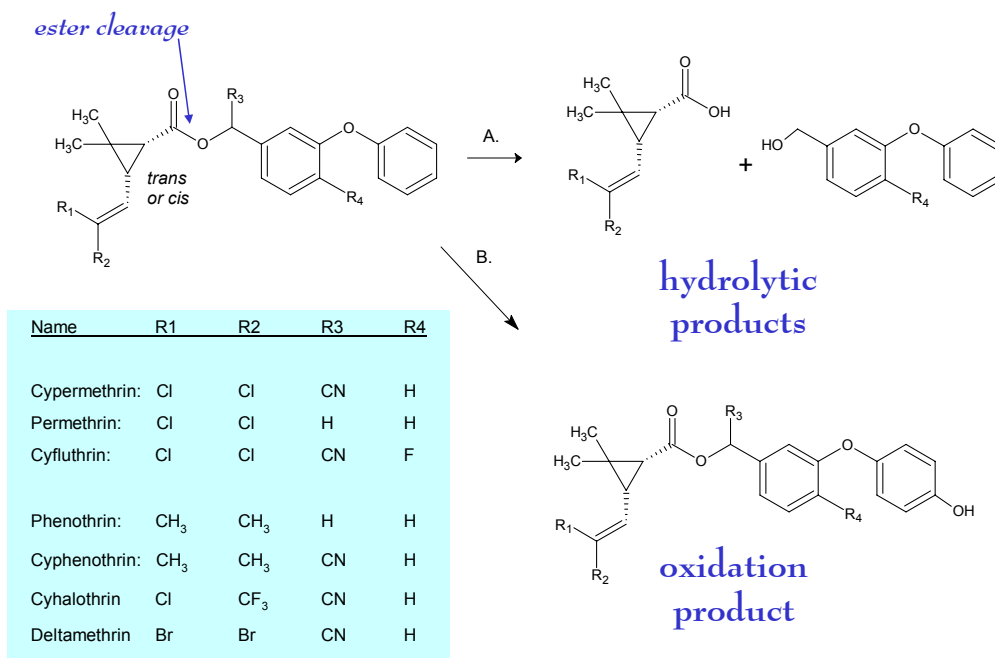
One question regarding distribution of pyrethroids to the central nervous system (CNS), the proposed target area, is if there is differential distribution of parent pyrethroid within the brain (see section 2.D.7 for more discussion). Anadon et al. (1996) reported on the kinetics of deltamethrin in the rat. In six different brain regions, Anadon et al (1996) observed parallel tissue concentration-time course curves, but the curves differed by 100-fold in concentration (medulla oblongata vs. hypothalamus). In contrast, Gray and Rickard (1981) and Rickard and Brodie (1985) reported no difference in the concentration of deltamethrin between brain regions in rats administered the compound intravenously. In other work by Anadon's laboratory with permethrin (1990) and lambda-cyhalothrin (2006), differences in tissue concentration were less than 10-fold across similar brain regions. The inconsistencies in the distribution of deltamethrin in brain raise questions if the deltamethrin results reported by Anadon et al. (1996) are accurate. Attempts at pharmacokinetic modeling the Anadon et al (1996) deltamethrin studies suggest that the differences in distribution of deltamethrin in rat brain would require the inclusion of significantly different regional brain blood flow rates. These differences in the blood flow rates would not be needed for permethrin and lambda-cyhalothrin. It is unlikely that there are such differences in regional brain blood flow rates that could account for this wide distribution of deltamethrin in this organ. Data on the concentration of deltamethrin in other tissues from Anadon et al (1996) were fit reasonably well by the PBPK models (see section 3) and suggests that these data are more consistent with other data in the literature.

2.D.5. Metabolism

The metabolism of pyrethroids is an important determinant of their neurotoxic effect. There are no metabolic activation steps, as the parent pyrethroid is the most neurotoxic entity. Direct injection of pyrethroids into the brains of mice (Lawrence and Casida, 1982) and rats (Gray and Rickard, 1982) results in the same neurotoxic effects as observed following intravenous administration of higher doses of these pyrethroids. In addition, tremors are not observed in rats following intravenous administration of hydrolyzed products of resmethrin (White et al., 1976). These observations suggest that the metabolism of pyrethroids is a detoxication step. Pyrethroids can undergo hydrolysis, oxidation or both metabolic steps (Soderlund et al., 2002) (Figure 2.4). Hydrolysis occurs across the ester bond between the acid and alcohol moieties and is catalyzed by hepatic carboxylesterases in rats and humans and serum esterases in rats (Anand et al., 2006; Nishi et al., 2006; Godin et al 2006,

Ross et al. 2006, Crow et al., 2007). Activity of human serum esterases towards pyrethroids appears to be minimal at best. Hydrolytic products of *trans*-permethrin are 3-phenoxybenzyl alcohol and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane. Both of these metabolites can be directly conjugated by Phase II enzymes, or oxidized and conjugated. Cytochrome P450 (CYP450) catalyzed oxidation of pyrethroids can occur on substituents attached to the cyclopropane ring of the acid moiety and phenoxybenzyl rings of the alcohol moiety. These hydroxylated metabolites may be conjugated, or hydrolyzed and conjugated. Metabolites from both pathways are then excreted primarily in urine.

Figure 2.4: Scheme of the hydrolytic (A) and oxidative (B) biotransformation of pyrethroids, for those pyrethroids with structure based on chrysanthemic acid and phenoxybenzoic acid moieties



EPA has examined differences in rat and human metabolism of the pyrethroid pesticides deltamethrin, esfenvalerate, *cis* and *trans*-permethrin, cypermethrin, cyfluthrin, bifenthrin, resmethrin and bioallethrin in liver microsomes and purified enzyme preparations (Appendix B)(Godin et al., 2006, 2007). This data set was developed, in part, to derive metabolic parameters for pyrethroid metabolism for use in PBPK models. The hepatic clearance of bifenthrin, bioallethrin, and *cis*-permethrin is much faster in rat microsomes than in human microsomes. Resmethrin and *trans*-permethrin hepatic clearance in human microsomes occurs at rates similar to or faster than in rat microsomes. *Cis*- and *trans*-permethrin clearance rates were slightly altered by combining them in a 40:60 ratio of *cis:trans*, consistent with commercially available products. The clearance of *trans*-permethrin in human microsomes was slower in the presence of *cis*-permethrin and may be an indication of competitive inhibition. These results are more fully presented in Appendix B.

In addition to determining the rate of microsomal metabolism, the enzymes involved were characterized by Godin et al. (2007) and Scollon et al. (2006) (in Appendix B). The primary mechanism for clearance of most pyrethroids in human and rat hepatic microsomes is NADPH dependent. Bioallethrin, cyfluthrin and bifenthrin were solely metabolized by this oxidative mechanism in rat and human hepatic microsomes. Resmethrin and *trans*-permethrin have additional non-NADPH dependent clearance mechanisms in hepatic microsomes from rats and humans. However, in human liver microsomes, of those pyrethroids tested, only resmethrin and deltamethrin appear to be cleared solely through non-NADPH dependent mechanisms (Godin et al., 2006; Scollon et al. 2006; see Appendix B). Pyrethroid oxidation occurs at several sites in the acid or alcohol moieties. Recent *in vitro* studies by Godin et al. (2007) and Scollon et al (2006; see Appendix B) show that rat CYP450 isoforms 1A1, 2C6, 2C11 and 3A2 have activity towards the different pyrethroids (Appendix B). Human CYP450s involved in the metabolism of pyrethroids include 2C8, 2C9, 2C19, 3A4 and 3A5. CYP450s 2C8, 2C9, and 2C19 have the most activity. The human esterases involved in the hydrolysis of pyrethroids are human carboxylesterase-1 (hCE1) and human carboxylesterase-2 (hCE2) with the former enzyme having more activity than the latter (Ross et al., 2006; Godin et al., 2006).

Understanding the enzymes involved in the metabolism of pyrethroids aids qualitatively and quantitatively in uncertainty and variability analysis of the PBPK models. Qualitatively, if the rat and human enzymes metabolizing the pyrethroids are the same, this increases the confidence of the rodent model for use in extrapolating metabolic data. In those cases where the enzymes involved are different between the species, species extrapolation may become less certain. Variability of metabolism rates can be related to changes in expression of these metabolizing enzymes due to gender, life stage and disease states. Information on changes in CYP450 expression is available for different life stages and disease states. More limited data are available on the variability of hCE1 expression in humans. The information on variability of expression of

metabolizing enzymes can be used in the PBPK models to estimate human variability in the relationship between exposure and dose.

2.D.6. Excretion

Following systemic absorption, pyrethroids are metabolized, conjugated, and excreted, primarily in the urine. In rats administered cypermethrin orally (2 mg/kg), 50% or more of the dose was excreted in urine 24 hr post-exposure (Crawford et al., 1981a). With increased dose of cypermethrin (200 mg/kg), a greater per cent of the dose was excreted in feces. Biliary excretion of pyrethroids appears to be a minor process as shown in studies with cypermethrin. Crawford et al. (1981a, b) reported that <2% of the administered dose of cypermethrin was eliminated by this route 4-5 hr post-administration. The biliary metabolites were glucuronide conjugates of the hydrolyzed parent (Crawford et al., 1981a, b). Pyrethroids that are eliminated in the feces are primarily parent compound (Crawford et al. 1981b), which appears to represent unabsorbed parent. However, Mirfazaelian et al. (2006) have suggested that exsorption may occur with the pyrethroids. This is the process whereby lipophilic compounds diffuse from the blood into the gastrointestinal tract and are excreted. However, this data should be interpreted cautiously compared to environmental exposures. At doses of 200 mg/kg it is likely that less of these highly lipid soluble chemicals are absorbed due to insolubility in the aqueous environment of the gut. Rats administered [¹⁴C-cyano]-cypermethrin and -fenvalerate exhale a low level of ¹⁴CO₂ (<5% of the dose) (Crawford et al., 1981a; Ohkawa et al., 1979). Much less ¹⁴CO₂ is exhaled when cypermethrin is labeled in the alcohol or acid moieties (Crawford et al., 1981a).

2.D.7. Animal Toxicokinetics

Anadon et al. (1991, 1996, 2006) have extensively examined the kinetics of three pyrethroids in rat. Permethrin, deltamethrin, and λ-cyhalothrin were administered orally or intravenously at toxic levels. While no fatalities were reported, the animals displayed the signs of Type I (permethrin) and Type II (deltamethrin, λ-cyhalothrin) pyrethroid toxicity. The doses were chosen so that parent and selected metabolites could be detected in tissues over a 24-48 hr period using an HPLC-UV method. In these studies, the animals were sacrificed serially and several tissues were removed and analyzed. Permethrin was administered as a mixture of the *cis:trans* (25:75) isomers. This mixture consists of 4 stereoisomers. However, with their HPLC method, Anadon et al. (1991) could only separate and quantify the diastereomers (*cis* and *trans*). These quantities were summed and the tissue data was reported as total permethrin. A drawback of this analysis is that the *cis*- and *trans*-isomers are metabolized at different rates. Since *trans*-isomers are more readily hydrolyzed, the proportion of the isomers present at any one time may change. They did, however, report the metabolites which result from the hydrolysis of *cis*- and *trans*-permethrin. These metabolites included 3-phenoxybenzyl alcohol and 3-phenoxybenzoic acid. The latter metabolite appears to be formed by two subsequent oxidations

by alcohol dehydrogenase (of 3-phenoxybenzyl alcohol) and aldehyde dehydrogenase (of 3-phenoxybenzaldehyde) (Choi et al., 2001).

For the three pyrethroids examined by Anadon and colleagues, the plasma profiles were characterized using classical pharmacokinetics by a two-compartment open model. Several toxicokinetic parameters for each of the compounds are displayed in Table 2.5. Overall, the kinetics for permethrin and λ -cyhalothrin were similar. They had rapid initial distribution phases and slower elimination phases. They also had similar absorption rate constants and similar distribution and elimination half-lives. The major differences in parameters were the area under the curve (AUC) and the maximum plasma concentration (C_{max}) where the values for permethrin were about 3-4 fold greater. Deltamethrin plasma kinetics was much different from the other two pyrethroids. The elimination half-life of deltamethrin for both the oral and intravenous routes was three times longer, the volume of distribution (V_d) 3-25 times greater, and the AUC and bioavailability significantly lower. The plasma kinetics for 4'-HO-deltamethrin was similar to those of deltamethrin.

Table 2.5: Plasma profile kinetics of permethrin, deltamethrin, and lambda-cyhalothrin in the rat following oral or intravenous administration

Parameter	Permethrin		Deltamethrin		Lambda-Cyhalothrin	
	Oral (460 mg/kg)	IV (46 mg/kg)	Oral (26 mg/kg)	IV (1.2 mg/kg)	Oral (20 mg/kg)	IV (3 mg/kg)
t _{1/2α} (h)	4.85	0.46	2.1	1.39	1.88	0.11
t _{1/2β} (h)	12.37	8.67	38.5	33.0	10.27	7.55
K _a (h ⁻¹)	0.76	-	1.14	-	0.8	-
t _{1/2a} (h)	0.91	-	0.61	-	0.87	-
V _d (l)	1.7	0.72	6.22	5.33	0.18	0.14
V _{dss} (l)	1.0	0.65	-	2.04	-	0.11
AUC (mg/hr l)	965	159	6.69	2.14	221.7	47.51
F (%)	60.69	-	14.43	-	67.37	-
MRT (h)	17.77	11.19	41.29	18.23	14.4	8.55
CL (l/h)	0.058	0.058	0.11	0.11	0.012	0.012
C _{max} (μg/ml)	49.46	-	0.46	-	15.65	-
T _{max} (h)	3.52	-	1.82	-	2.69	-

Using classical pharmacokinetics, Anadon and colleagues fit a one-compartment model to the permethrin and deltamethrin data and a two-compartment model for λ -cyhalothrin (Anadon et al., 1991, 1996, 2006). Table 2.6 shows the brain, sciatic nerve, and liver tissue kinetics of the pyrethroids analyzed by Anadon and colleagues. Due in part to their lipophilic nature, the pyrethroids distributed from plasma to nervous tissue. However, the proportion that distributed into the brain varied significantly among the compounds. Of the three pyrethroids, λ -cyhalothrin distributed the least into nervous tissue (shown by the ratio of the $AUC_{tissue}/AUC_{plasma}$). Deltamethrin distributed the greatest into the nervous tissue, with an AUC ratio that ranged from 2 to almost 300. Once the chemicals were in the tissues they left slowly, as shown by the elimination half-lives, which ranged from a low of 10 hr in the cerebellum for permethrin to a high of 41 hr in the hypothalamus for deltamethrin.

Table 2.6: Selected tissue kinetic values following oral administration of permethrin, deltamethrin, or lambda-cyhalothrin in the rat

Tissue	Permethrin		Deltamethrin		Lambda-cyhalothrin	
	$AUC_{tissue}/AUC_{plasma}$	Elimination $t_{1/2}^a$ (hr)	$AUC_{tissue}/AUC_{plasma}$	Elimination $t_{1/2}^b$ (hr)	$AUC_{tissue}/AUC_{plasma}$	Elimination $t_{1/2}^c$ (hr)
Hypothalamus	3.48	12.6	295.3	40.76	1.99	34.82
Cerebellum	1.16	9.9	34.78	33.0	1.13	15.82
Frontal Cortex	4.27	13.86	8.84	26.65	1.26	18.82
Hippocampus	3.71	23.1	92.99	38.5	0.98	23.81
Medulla Oblangata	1.9	22.36	2.32	23.89	1.00	22.42
Sciatic Nerve	8.7	16.27	- ^c	-	2.27	20.12
Liver	0.44	16.5	- ^c	-	0.92	13.14

^aelimination half-life derived from a one compartment model

^belimination half-life derived from a two compartment model

^ctissue not analyzed

2.E. Human Pharmacokinetic Data

There are only a few published studies of individuals intentionally exposed to pyrethroids (Table 2.7). These studies are expected to be used in the PBPK modeling efforts primarily in calibration of absorption parameters for the oral, dermal, and inhalation routes of exposure.

In the published literature, cypermethrin is the most-studied pyrethroid with human volunteers (Eadsforth et al., 1988; Woollen et al., 1992). In a metabolism study with cypermethrin (1:1 *cis/trans* mixture), the subjects excreted 78% of the *trans* isomer dose and 49% of the *cis* isomer dose in urine as the cyclopropane carboxylic acid metabolite within 24 hr (Eadsforth et al. 1988). This shows the importance of hydrolysis in the elimination of cypermethrin in man. The elimination of metabolites was also dose-related (increased administered dose, resulted in increased urinary metabolites detected). Following an acute oral dose of *alpha*-cypermethrin (*cis* isomer), the subjects excreted within 24 hours 43% of the *cis* isomer dose in urine, and in repeated dosing 49% of the dose. These data suggest that the kinetics of the *cis* and *trans* isomers are independent in this dose range and that cypermethrin does not tend to bioaccumulate. In a metabolism study with pyrethrin I, a structural analog of cyclopropane-bearing pyrethroids, the subjects excreted 60% of the dose urine as the cyclopropane carboxylic acid (Leng et al. 2006). Dermal absorption studies with human subjects are available for cypermethrin and permethrin (Eadsforth et al., 1988; Woollen et al., 1992; Tomalik-Scharte et al., 2005) which support the *in vitro* findings that only small amount of pyrethroids are available to the systemic circulation following dermal exposure. Leng et al. (1997) conducted a study to evaluate excretion of metabolites in male volunteers exposed to air concentrations of cyfluthrin of 160 $\mu\text{g}/\text{m}^3$ for 10-60 min. Following this air exposure, 93% of the metabolites were excreted within 24 hr.

Table 2.7: Human Dose-Excretion Studies

	Human Studies	Subjects (N)	Exposure	(s)ingle (r)repeat	t _{1/2} (h)	Excreted(%)			Refs
						total	cis	trans	
Dermal									
permethrin	IC,PK, IRB, ANP	6	215 (mg), healthy	s	32.7 h (total)	0.35% (168h, DCCA)			1
permethrin	IC,PK, IRB, ANP	6	215 (mg), scabies	s	28.8 h (total)	0.47% (168h, DCCA)			1
permethrin	IC,PK, IRB, ANP	6	60 (g) cream	s	37.8 h (total)	0.52% (168h, DCCA)			1
cypermethrin	IC,PK, IRB, ANP	2	25 mg / 50 cm ²	s		0.1% (72h, DCCA)			2
cypermethrin	IC,PK, IRB, ANP	6	31 mg / 800 cm ²	s	13 h (total)	0.3% (120 h, DCCA)			3
cypermethrin	IC,PK, IRB, ANP	6	31 mg / 800 cm ²	s	13 h (total)	1.2% (120 h, PBA)			3
Oral									
			(mg)						
alphacypermethrin	IC,PK, IRB, ANP	6	0.25-0.75	s			43% (24h, DCCA)		2
alphacypermethrin	IC,PK, IRB, ANP	6	0.25-0.75	r			49% (24h, DCCA)		2
cypermethrin	IC,PK, IRB, ANP	6	0.25-1.5	r			45% (24h,DCCA)	75% (24h,DCCA)	2
cypermethrin	IC,PK, IRB, ANP	6	3.3	s	16.5 h (total)		19% (120h, DCCA)	36% (120h, DCCA)	3
Inhalation									
			□micro-g/m ³						
cyfluthrin	IC,PK, IRB, ANP	9	160 (1 h)	s	6.9 h (c-DCCA) 6.2 h (t-DCCA) 5.3 h (FPBA)	93% (24 h)			4
<p>Abbreviation: IC, Informed Consent; PK, Pharmacokinetic endpoint; IRB, Institutional Review Board/ Ethisc Committte; ANP, Adult, Non-pregnant women</p> <p>References:</p> <p>1 Tomalik-Scharte et al. (2005)</p> <p>2 Eadsforth, Bragt, and Sittert (1988)</p> <p>3 Woollen et al. (1992)</p> <p>4 Leng et al. (1997)</p>									

2.F. Dose Metric

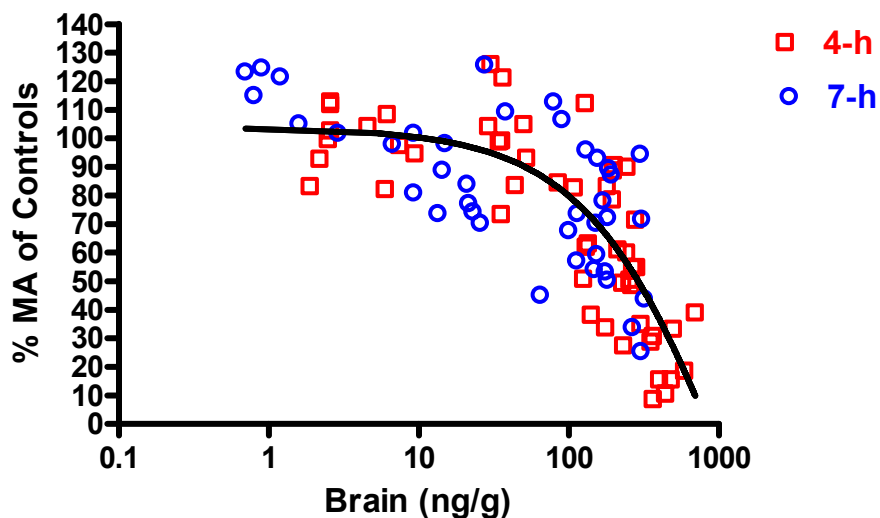
Dose can be expressed in a variety of metrics such as a daily intake (mg/kg/day), blood or tissue concentrations, AUC and others. In the evaluation of the toxicity of a chemical, one of the most difficult tasks is determining which dose metric to use. This choice has many impacts from data collection to the structure of physiologically based pharmacokinetic models. A dose metric is useful in aiding extrapolations across species and exposure scenarios. Ideally, an appropriate dose metric should reflect both the magnitude and frequency of exposure, and it should be clearly related to the toxic endpoint of concern by a well-defined mechanism. However, this is often difficult, because human exposures may be very different from the highly controlled exposures in animal experiments. Even comparable exposures may be followed by very different pharmacokinetics (absorption, distribution, metabolism, and/or elimination) in animals and humans. Finally, the sequelae of exposure in the form of a variety of responses related to age, organ, and species sensitivity complicate the choice of a common dose metric. Despite these complexities, relatively simple default approaches, have often been recommended (U.S. EPA, 1992a, 1996) for species extrapolation such as body surface or body weight scaling of daily exposures.

Brodie and Reid (1967) suggest that the response to a drug is determined by the amount bound to its biological receptor, and since the drug-receptor complex is in dynamic equilibrium with the free drug in the plasma, the biological response of a drug will be related to its plasma concentrations. There is no reason to believe that this relationship will not be true for pyrethroids. Limited studies have examined the relationship between blood or brain concentrations and neurotoxic effects of pyrethroids. The concentration of deltamethrin (administered ip), cismethrin (administered iv), and bioresmethrin (administered iv) in brain of rats was correlated with the induction of the CS-syndrome for deltamethrin and the T-syndrome for cismethrin and bioresmethrin (Gray et al., 1980; Rickard and Brodie, 1985; White et al., 1976). For each pyrethroid, a chemical specific threshold level in brain was required for the neurobehavioral effects (tremors for bioresmethrin and cismethrin, tremors and choreoathetosis for deltamethrin) to develop. These levels were 2.5 – 3 nmole/g for deltamethrin, 3.5 nmol/g for cismethrin and 14.5 nmole/g for bioresmethrin. Similar correlations were also observed between blood and effect in rats treated intraperitoneally with deltamethrin (Rickard and Brodie, 1985).

The dose response relationships for the neurotoxicity of deltamethrin in adult animals are similar in acute and chronic exposures (Nemec, 1998a, 1998b). These data suggest that for the neurotoxicity, momentary brain and/or blood concentrations may be useful dose metrics for pyrethroid exposures. Efforts at EPA have examined the hypothesis that blood and brain pyrethroid concentrations are predictive of neurotoxic effects for pyrethroids (Appendix C). Briefly, the dose response for decreased motor activity and blood and brain concentrations for bifenthrin were determined at 4 and 7 hr post-exposure. Bifenthrin exposure decreased motor activity

in a dose-dependent manner at both time points. A Hill model was fit to the tissue concentration vs behavior data. The relationship between whole blood concentrations and motor activity was different between the 4 hr and 7 hr groups with the ED_{50} and slope factor for the 4-hr (85 ng/g and 3.1, respectively) greater than the ED_{50} and slope factor for the 7-hr (41 ng/g and 0.5, respectively). Model fits to the brain concentration vs behavior data were not different between the time points and therefore combined for an estimated ED_{50} of 278 ng/g with a slope factor of 1.6 (Figure 2.5). The difference between the relationship between blood and brain concentrations and behavioral alterations may be that the blood concentrations are more dynamic than the brain concentrations. However, with the limited time course examined in this study it is difficult to quantitatively support this statement and further time course data is needed.

Figure 2.5: The relationship between brain concentrations and decreased motor activity (MA) in rats exposed to bifenthrin



The early studies by White et al (1976), Gray et al. (1980), and Rickard and Brodie (1985) and the newer studies performed by the EPA indicate a relationship between increasing brain concentrations and increasing behavioral effects. In fact, it has been proposed that a threshold brain pyrethroid concentration is required for animals to display signs of the T- and CS-syndromes (White et al., 1976; Gray et al., 1980; Rickard and Brodie, 1985). Since these data indicate that the higher the concentration in the brain, the greater the neurobehavioral effect, it is likely that peak concentration would be of greatest concern. Further support for peak concentration is the demonstration that the dose response for pyrethroid neurotoxicity is similar in acute and chronic exposures. In the development of PBPK models for pyrethroids, these data demonstrate that model predictions of brain concentrations may be used to predict neurobehavioral effects of pyrethroids.

The experiments examining the dose response relationship between tissue concentrations and neurobehavioral effects have only examined a limited number of pyrethroids with limited dose and time response relationships. These studies have not examined alternative dose metrics such as AUC. Further research at EPA will examine additional dose metrics with several additional pyrethroids and behavioral effects.

3. Generic Pyrethroid PBPK Model Structure

Advances in the understanding of mode of action and pharmacokinetics allow for the development of more sophisticated models as alternatives to some of the default approaches. The following discussion describes approaches to developing physiologically-based pharmacokinetic models of pyrethroid pesticides for future use in risk assessments.

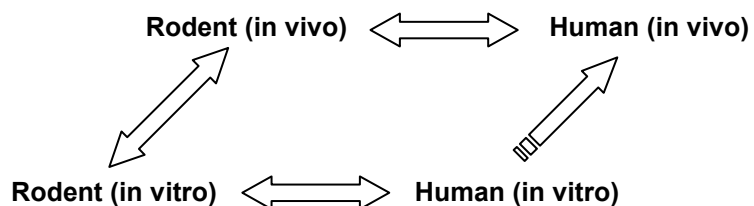
Since it is also our goal that predictive methods should be applicable to existing and emerging pyrethroids prior to any animal experiment is conducted, the Agency is interested in establishing a generic PBPK model for this class, independently of any specific pyrethroid. The properties of the pyrethroid that determine its overall kinetic properties could then be determined through the use of separate *in vitro* surrogates of the important absorption, distribution, metabolism, and elimination (ADME) properties. Coupled with *in vitro* toxicity data, simulation of the overall biological effect *in vivo* could become feasible.

3.A. Modeling Goals

The acute neurotoxic effects of the pyrethroids have been studied to a large extent in insects and rodents (Soderlund et al., 2002, Shafer and Meyer 2004; Wolansky et al., 2006). However, quantitative models linking exposure to effects in animals and human are not available. The practical considerations of animal toxicity studies require animal testing at high exposure levels, usually by a single route of administration, and with a simplistic exposure pattern. The key to successful extrapolation to humans is the reliability of target-tissue dose estimations across species (Andersen, 2003). PBPK models allow calculations of tissue doses of chemicals and their metabolites over a wide range of exposure conditions (Chiu et. al., 2007; Chiu and White 2006; Keys et al., 2003). By incorporation of anatomy and biological processes, the models allow adaptation to different organisms (Medinsky and Valentine, 2001). Because the rate constants in PBPK models represent known or hypothesized biological processes, they may be acquired using *in vitro* models of the relevant processes.

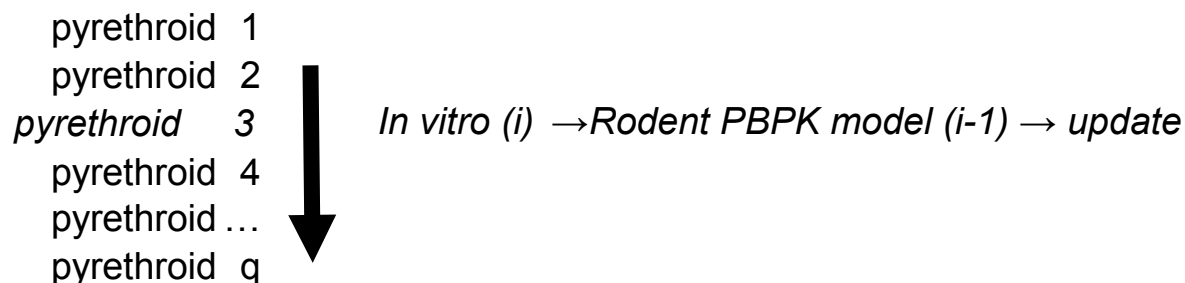
An accepted approach to conduct species extrapolations is through a parallelogram approach. Although the roots of the parallelogram approach are in genetic toxicology (Sobels, 1993; reviewed in EC/US Workshop, 1995), it has been applied to species extrapolation of immune function and resistance (Goettsch et. al., 1998) and to evaluate the risk of developmental neurotoxicity posed by methylmercury (Lewandowski et al., 2003). The parallelogram approach has also been incorporated within a PBPK framework to estimate the health risks of exposure to structurally related chlorofluorocarbons (Jarabek et al., 1994). Based on considerable evidence that the mechanistic determinants of disposition are common among the pyrethroids, a parallelogram approach is proposed (Figure 3.1). The steps are as follows:

Figure 3.1: Parallelogram approach for extrapolation of pharmacokinetics



- 1) First, a hypothesis based model is developed that includes descriptions of the proposed determinants of disposition in the rodent. These are the key processes that govern absorption, distribution, metabolism, and excretion. In the case of pyrethroids where physicochemical properties are relatively similar across the class a family modeling approach may be applied. In this approach a single model structure may be applied to all pyrethroids with similarities in some constants expected (partition coefficients, coefficients of permeability). Where the pyrethroids interact with an enzyme, transporter, or receptor, a divergence in the specific governing constants is expected (e.g., the rate constant of hydrolysis and oxidation).
- (2) Second, through PBPK modeling evaluations in the rodent, constants that are largely invariant as additional pyrethroids tested are identified and calibrated. Likewise, processes that are compound-dependent are identified and examined in the pertinent *in vitro* system. The ability to properly scale and incorporate the derived constants in the PBPK model is evaluated. Predictions that depart from the data suggest that the biological process has either not been properly captured (e.g., transporter) or represented (e.g., a bias in enzyme titer). In Figure 3.2 below, the model structure and “invariant parameters” are based on pyrethroids 1 and 2; this represents the *a priori* model for pyrethroid 3.

Figure 3.2: Scheme for iterative parameterization and testing of generic pyrethroid PBPK model



- 3) The human *in vitro* data (e.g., Godin et al., 2006) is then incorporated in the PBPK model with the pertinent human anatomical/physiological data. Empirical adjustments for *in vitro* to *in vivo* scaling derived from the mammalian surrogate are applied, establishing the human PBPK model. To conduct the risk assessment, population distributions of exposure, either measured or modeled (e.g., SHEDS) are interpreted by the human PBPK rendering population distributions of blood and brain concentration, dose-metrics pertinent to peripheral nervous system (PNS) and CNS effects. The dose metrics are interpreted based on dose response relationships understood in the rodent system.

The process of model development described here leads to an important organizing principle that can enhance our confidence in the pyrethroid models. Using a family modeling approach (Steps 1 and 2 above) allows for hypothesis based pharmacokinetic studies. The rat PBPK model, with the appropriate *in vitro* data pertaining to the next pyrethroid, can be used to design pharmacokinetic studies. If the PBPK model predicts the pharmacokinetics of the new pyrethroid our confidence in the model increases. The family model approach allows for the assessment of the overall model structure with each iteration. The more iterations through this process, the more confidence in the models predictive abilities. Thus, the rat deltamethrin model is not only assessed by data from deltamethrin, but is evaluated by model fits to data for every other pyrethroid. As our confidence in the family model increases across pyrethroids, our confidence in the use of this modeling approach for rodent to human extrapolation (Step 3 above) also increases.

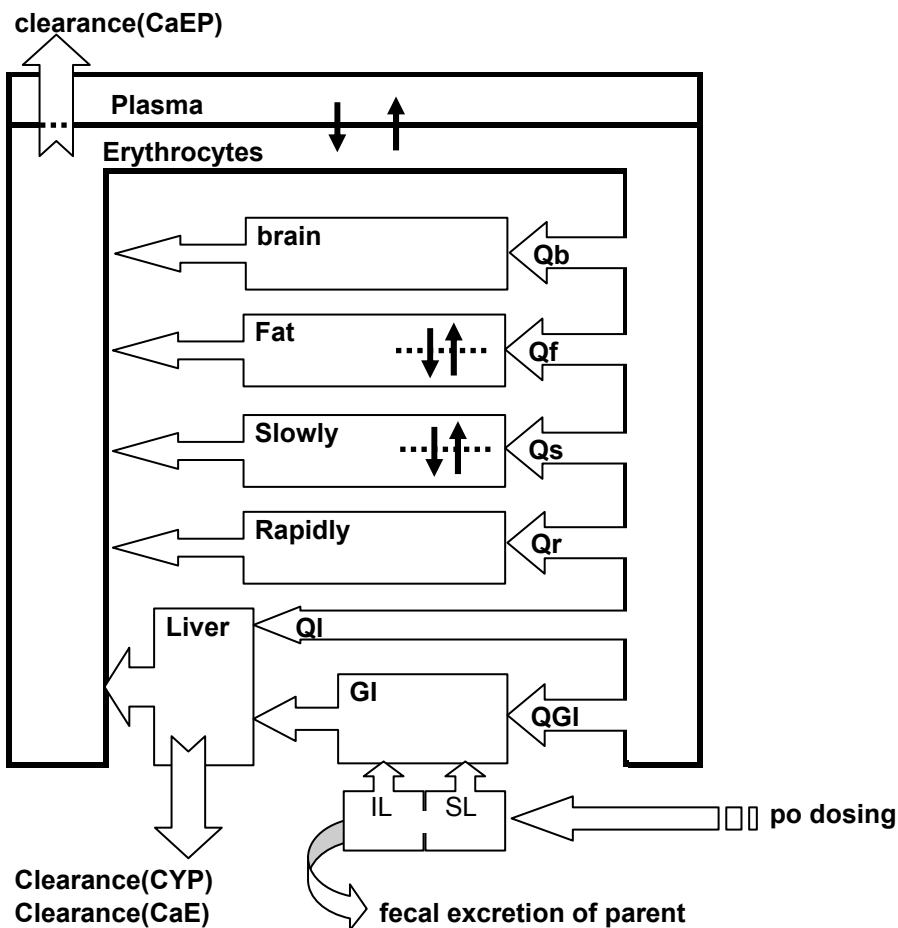
A PBPK model for pyrethroid pesticides should include compartments that are important for the absorption, distribution, metabolism, elimination, and toxicity. Oral, dermal and inhalation are routes of exposure thus the gastrointestinal tract, lungs and skin are important compartments for the model. Liver and adipose tissue should be included as organs involved in the metabolism and distribution of pyrethroids, respectively. The effects of pyrethroids on the central nervous system appear to mediate the neurotoxicity and momentary brain concentrations appear to be a useful dose metric. There is suggestive evidence that the PNS is also involved in the neurotoxicity. Based on our understanding of the mode of action, the model should include a CNS, PNS, and blood compartments. The PNS is a relatively diffuse system and, blood concentrations may be an adequate surrogate for this tissue. Thus, the Agency's modeling efforts include the blood and CNS compartments. In the future, additional compartments could be included based on further exposure, pharmacokinetic, and toxicological data as well as model evaluation.

The following section outlines the modeling approaches used in the development of a generic PBPK model for pyrethroids. This outline starts with a relatively simple pyrethroid, deltamethrin, and expands to include pyrethroids that are more complex. There are over 20 pyrethroids registered in the US. PBPK models will not be developed for all pyrethroids. Modeling efforts, both rodent and human, will be prioritized based on those compounds for which there is greatest dietary and residential exposure and for those with significant risk [exposure prevalence x potency].

3.B. PBPK Model Of Deltamethrin Disposition In The Rat ('Delta Model')

As a class, pyrethroid pesticides offer a number of challenges for PBPK modeling. Initial modeling efforts have focused on deltamethrin because it is readily available as a single stereoisomer. Because of similar physiochemical and pharmacokinetic properties of pyrethroids, the development of a PBPK model for deltamethrin is expected to provide the basic framework for the development of a generic PBPK model for all pyrethroids. Recently Mirfazaelian et al. (2006), in collaboration with EPA's National Exposure Research Laboratory (NERL), developed a PBPK model of deltamethrin disposition in the rat (hereafter referred to as the "Delta Model"). Since pyrethroids are metabolized by common pathways in rodents and humans (Godin et al., 2006; Soderlund et al., 2002; Appendix B), the model provides a framework to extend the quantitative description of disposition to other pyrethroids in rats and humans. The Delta Model was constructed for the adult male Sprague-Dawley rat and employs both flow-limited (brain, gastrointestinal [GI] tract, liver, and rapidly perfused tissues) and diffusion-limited (fat, blood/plasma, and slowly perfused tissues) compartments (Figure.3.3). In contrast with PBPK models of volatile organic chemicals, which often employ flow-limited kinetics, deltamethrin required implementation of diffusion-limited compartments to simulate the slower uptake and clearance, in particular for the fat compartment.

Figure 3.3: Schematic of PBPK model for deltamethrin in rats (Mirfazaelian et al., 2006). Delivery of deltamethrin to the brain, liver, GI tract, and rapidly perfused tissues is flow limited, while delivery to the remaining tissues (erythrocytes, fat, and slowly perfused tissues) is diffusion limited. QGI represents flow of the plasma through the GI tract to the liver. CYP and CaE represent metabolic clearance

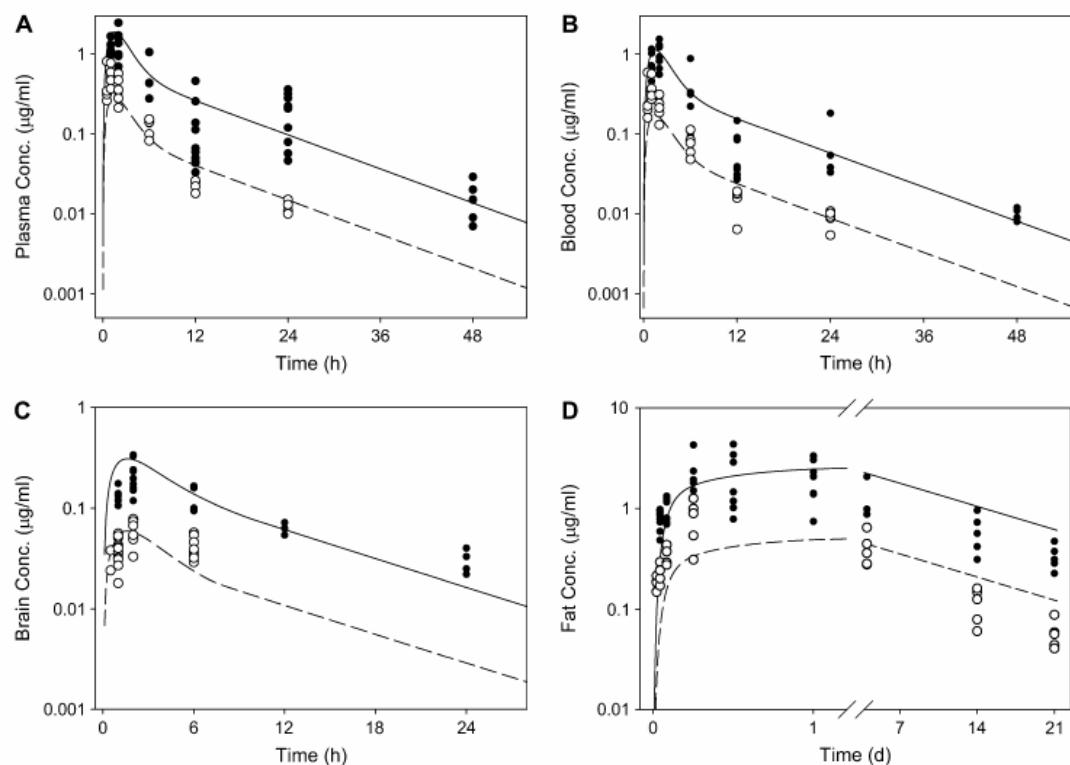


Once absorbed, pyrethroids undergo oxidative metabolism via CYP450 enzymes in the liver and hydrolysis via carboxylesterases in the liver and plasma (Soderlund et al., 2002; Soderlund and Casida, 1977; Casida et al., 1983; Gray and Soderlund, 1985). These processes were incorporated in the mass-balance equations of the Delta Model. Anand et al. (2006) assessed the contribution of each pathway by measuring the rates of disappearance of the parent compound upon incubation of a series of concentrations of deltamethrin with adult Sprague-Dawley rat plasma and liver microsomes. K_m and V_{max} values were derived for each metabolic pathway, scaled, and incorporated into the PBPK model. To account for tissue distribution, experimental plasma and tissue time-course from rats of 10 days age post natal were used to estimate the plasma:tissue partition-coefficients (PC). The tissue:plasma PCs were calculated as the ratio of the AUC of deltamethrin in tissue and plasma. Anatomical information on organ volumes and flow rates was applied from Brown et al. (1997), Delp et al. (1991), and Schoeffner et al. (1999) for 70 day old rats.

The model was evaluated with experimental data from the adult male Sprague-Dawley rat dosed with 2 or 10 mg/kg deltamethrin in a dosing solution of glycerol formal (1 ml/kg). Groups of five rats were sacrificed 1, 2, 6, 12, 24, and 48 hr after dosing and arterial blood, whole brain, and perirenal fat were collected at each time point. An additional group of adult animals was utilized for an iv experiment in which the rats received 1 mg/kg iv. In both experiments, blood samples were centrifuged for plasma separation. These data were also employed in model development to parameters, which were otherwise not directly measurable, such as the permeability area cross product pertinent to the diffusion-limited compartments and parameters related to GI absorption.

The details of the model are presented in Mirfazaelian et al. (2006) and in Appendix D. Overall, the model adequately describes the experimental data in each biological medium (Figure. 3.4). A sensitivity analysis performed by the investigators revealed that the metabolic constants of oxidative and hydrolytic metabolism are among the most important determinants of blood and brain-related dose-metrics. Mass balance analysis revealed that metabolism accounts for 83% and 91% of the clearance of 2 and 10 mg/kg doses, respectively. Hydrolysis in the plasma was not among the more sensitive parameters and this is reflected in its impact on overall clearance (~8% for 2 and 10 mg/kg). Other parameters of influence were those related to GI absorption and excretion. The plasma:liver PC was important with respect to blood and brain dose metrics, while the plasma:brain PC was important only to the brain. Interestingly, the volume of the muscle compartment and coefficient of permeability were important determinants of peak blood and brain concentration. Both the fat and muscle compartments have large and comparable contributions to the steady-state volume of distribution (V_{dss}). Each comprises about 40% of V_{dss} , the muscle owing to its large volume (78% BW; Mirfazaelian et al., 2006) and the fat owing to its high PC (49; Mirfazaelian et al., 2006), where compartment contribution is approximately equal to the product of compartment volume x PC (Poulin and Theil, 2000).

Figure 3.4: Simulation and experimental time-course data for deltamethrin in (A) plasma, (B) blood, (C) brain, and (D) fat of rats receiving 2 (dashed line) or 10 mg/kg (solid line) by oral gavage. Filled and open circles represent deltamethrin measured in 10 and 2 mg/kg deltamethrin by oral gavage, respectively. (Reprint of Figure. 4 from Mirfazaelian et al., 2006).



3.C. Extension of the Delta Model To Other Pyrethroids

3.C.1. Overview

Based on similarities in their structure and log P, most pyrethroids are expected to possess similar physicochemical characteristics as deltamethrin. In general, for the pyrethroids, it is anticipated that some parameters such as tissue distribution will be similar. This is not the case for metabolic clearance. Following oral administration pyrethroids are absorbed about 60-90% depending on the vehicle of administration and reach peak levels in plasma in a few hours. The half-life in plasma is about 0.5 day to 1 day depending on structure. Clearance from the plasma is prolonged following dermal absorption, for which the overall level of absorption into the systemic circulation is low (about 2-5%) (Appendix A). Although they are highly lipophilic, (logP ~ 4-6) they tend not to store in the body, owing to the rapid rate in which they are metabolized and excreted. Only trace levels remain in the fat after a few days of exposure. Biotransformation is thus an important organizing principle in the pharmacokinetic modeling of the pyrethroids. The principle concern is to describe dose metrics that pertain to the parent compound.

Table 3.1 illustrates the impact of pyrethroid stereochemistry on rates of metabolism. The rates, abstracted from Soderlund and Casida (1977), were determined from the elimination of pyrethroids in mouse liver microsomes and are scaled relative to 1R, *trans*-resmethrin. The data show that rates of elimination of diastereomers vary much more in the esterase pathway than the oxidative pathway. Whereas the *trans*:*cis* ratio for the oxidative pathway ranges 0.7-1.8 (geometric mean=0.86), this ratio ranges from 0.4-110 (geometric mean=12.4) for the esterase pathway. For the total observed rate, the profile for diastereomers is somewhat dampened by the oxidative pathway; the ratio ranged from 0.92-13.92 (geometric mean=2.815). A limited amount of data was available to evaluate the influence of enantiomers (permethrin and resmethrin). In contrast to the diastereomers, the enantiomeric pairs do not display sharp difference in rates. Evaluating enantiomer pairs within their respective diastereomers, for the oxidative pathway the R/S ratio ranges from 1.0-1.8 (geometric mean=1.23); the esterase pathway ranges from 0.5-1.7 (geometric mean 0.82); and for the total pathway 1.2-1.8 (geometric mean= 1.4).

Table 3.1: Metabolism rates for pyrethroids in mouse liver microsomal enzymes: diastereomers and enantiomers

	Total (Obs)	Esterase	Oxidase	Oxidase	Esterase	total
<i>Diastereomers</i>		<i>Diastereomer Ratios (trans:cis)</i>				
Fluoresmethrin 1R, trans	181	143	8	0.8	50.6	13.92
Fluoresmethrin 1R, cis	13	<4	10			
Chloroethrin 1R, trans	180	155	21	0.9	109.6	13.85
Chloroethrin 1R, cis	13	<2	24			
Tetramethrin 1R, trans	95	25	62	0.8	8.3	1.02
Tetramethrin 1R, cis	93	3	75			
Phenothrin 1R, trans	78	59	27	0.7	20.9	2.11
Phenothrin 1R, cis	37	<4	37			
Bromophenothrin 1R, trans	36	20	15	0.8	14.1	1.89
Bromophenothrin 1R, cis	19	<2	18			
Cyanophenothrin 1R trans, alpha-RS	11	3	5	0.6	1.4	0.92
Cyanophenothrin 1R cis, alpha-RS	12	<3	8			
Cypermethrin 1RS, trans, alpha-RS	18	17	4	0.8	12.0	2.57
Cypermethrin 1RS, cis, alpha-RS	7	<2	5			
Esfenvalerate (S+), alpha-RS)	15	<2	11	1.8	0.4	1.36
Esfenvalerate (R-), alpha-RS)	11	4	6			
Resmethrin 1RS, trans	83.0	63	20	0.7	25.5	2.77
Resmethrin 1RS, cis	30.0	2.47	27.5			
Permethrin 1RS, trans	116.5	93	23.5	1.0	43.8	4.46
Permethrin 1RS, cis	26.1	2.12	24			
<i>Enantiomers</i>		<i>Enantiomer Ratios (R:S)</i>				
Resmethrin, 1R trans	100	79	20	1.0	1.7	1.4
Resmethrin, 1S trans	69	47	20			
Resmethrin, 1R cis	29	<3	29	1.1	0.8	1.1
Resmethrin, 1S cis	26	<4	26			
Permethrin 1R, trans	112	77	30	1.8	0.7	1.8
Permethrin 1S, trans	123	109	17			
Permethrin 1R, cis	29	<2	26	1.2	0.5	1.2
Permethrin 1S, cis	26	<4	22			

Source: Soderlund and Casida (1977). To determine ratios of rates that included values below limit of detection (LOD; e.g., "<3"), the LOD was replaced with LOD/ $\sqrt{2}$

3.C.2. Incorporation of Chiral Chemistry of Pyrethroids

PBPK models are of greatest utility in risk assessments when the toxicophore is clearly identified. The parent chemical mediates the neurotoxicity of pyrethroids. Metabolism is thought to be an inactivation step. The chiral chemistry of the pyrethroid pesticides complicates the identification of the toxicophore. Limited evidence indicates that stereochemistry of pyrethroids significantly influences their biological activity. As described above, most of the toxicity, pharmacokinetic and exposure data lacks stereochemistry resolution.

The chiral chemistry of pyrethroids can be incorporated into a PBPK model in several manners. Since most available data does not provide stereochemistry resolution, one could assume that all stereoisomers are equally potent and share the same pharmacokinetics. If the ratio of these stereoisomers is constant between the technical grade tested in experimental animals and their tissue concentrations and in human exposures, and their tissue concentrations, assuming all stereoisomers are equally potent is a reasonable assumption. However, the farther one gets from this condition, the greater the impact of this assumption on the risk estimate. Alternatively, one could model each individual isomer for every pyrethroid. A third approach could potentially focus on stereoisomers of a particular pyrethroid if they have sufficient biological activity, either as a toxicant or by altering the pharmacokinetics of the biologically active isomers. The last two alternatives are only practical if the data are available. It is likely that a combination of these three modeling approaches will be used depending on the pyrethroid. In building models, the resolution of the model will likely be dictated by the available data to build, evaluate, and use as inputs. The resolution of most toxicity, pharmacokinetic, and exposure data does not include enantiomer resolution.

3.C.3. Case Study: Permethrin

To evaluate the utility of a generic PBPK model for pyrethroids and the use of *in vitro* data in PBPK model development, pharmacokinetic evaluations for permethrin and cypermethrin are underway. Permethrin is one of the most widely used pyrethroids and is sold as a mixture of *cis* and *trans* stereoisomers. Both *cis*- and *trans*-permethrin are readily available and are easily separated and quantified using either HPLC or GC methods. Concentrations of both *cis* and *trans*-permethrin in food, surfaces, and air are regularly reported in the literature. Cypermethrin is a mixture of up to four diastereomer pairs. While these diastereomers can be separated through either HPLC or GC methods, only recently have limited quantities of purified stereoisomers become available. Thus, concentrations of cypermethrin in food and environmental samples are reported as total cypermethrin concentrations. Presently, only a preliminary model for permethrin has been developed; cypermethrin is not presented in this paper.

The Delta Model was elaborated for deltamethrin, a single isomer pyrethroid, in part to avoid difficulties in interpretation of mixture of isomers, common to the pyrethroids. To evaluate the feasibility of extending the model to other pyrethroids, a provisional model was developed to describe the pharmacokinetics of permethrin. Parameter values are indicated in Table 3.2. Intrinsic clearance was estimated from *in vitro* studies of the rate of disappearance of permethrin in rat liver microsomes (see Appendix B).

Table 3.2: Model parameters for deltamethrin and permethrin

	Deltamethrin^a	cis-Permethrin	trans-Permethrin	Permethrin
Stereochemistry	enantiomer 1R cis alpha S	cis-diastereomer 1:1 ratio 1R, 1S	trans-diastereomer 1:1 ratio 1R, 1S	mixture 40% cis, 60% trans
Partition Coefficient				
Brain:plasma	0.22	2.5	0.5	2.5
Liver:plasma	0.44	set	set	set
fat:plasma	48.9	set	set	set
slowly:plasma	5.6	set	set	set
rapidly:plasma	0.44	set	set	set
GI:plasma	0.44	set	set	set
RBC:plasma	0.17	set	set	set
Vdss (L/Kg) ^b	7.8	7.8	7.8	7.8
Diffusion-limited parameters; Tissue permeability cross-product area (L/h)				
Fat(PAF)	0.004	set	set	set
Slowly(PAS)	0.7	set	set	set
RBC(PAE)	1.0	set	set	set
Brain (PABRN)	0.001 ^c	0.001	1	0.001
Scaled <i>in vitro</i>				
Clearance Constants (L/h/kg^{0.75})				
Liver CYP	1.02	23.42 ^d	6.92 ^d	15.22
Liver CaE	0.34	0.0	12.44	3.83
Plasma CaE	0.04	0.13 ^e	0.8 ^e	0.53 ^e

Uptake rate constants (h⁻¹)				
Gastric (Ks)	0.01	set	set	set
Intestinal (Ki)	0.9	set	set	set
GI transfer rate	0.7	set	set	set
Fecal Elimination	saturable ^e	27% ^f	set ^f	set ^f

set: indicates that deltamethrin parameter value is adopted

^a Deltamethrin model parameters were obtained from Mirfazaelian et al. 2006

^b Vdss estimated as $(\sum V_x \cdot \lambda_{x:plasma}) + V_p$. The fat is ~ 47% Vdss, muscle ~ 42%Vdss

^c In Mirfazaelian et al. 2006, brain was modeled with flow limited kinetics

^d Clearance constants for oxidation required attenuation by a factor of 0.25

^d Estimated based on work of Ling-Jen Furguson (Unpublished)

^e Under revision

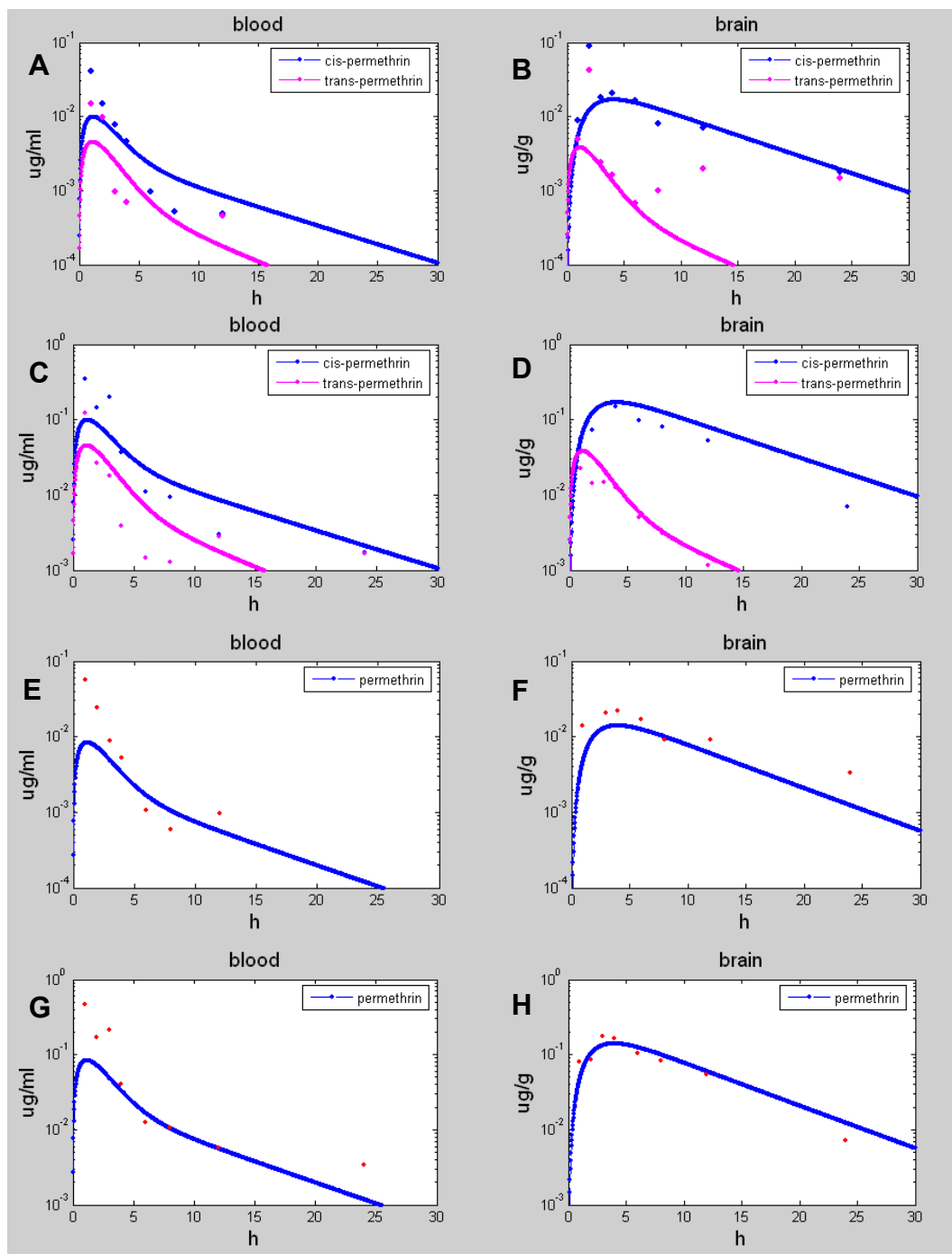
^f Estimated based on Ruzo et al. (1978), Bosch (1990), and Anadon (1991)

The model predictions were evaluated based on experimental data from 65-day old male Long-Evan rats exposed to a technical mixture of 40:60 *cis*- and *trans*-permethrin. Rats were exposed by gavage to a single dose of either 1 or 10 mg/kg of permethrin and sacrificed at 0.5 to 24 hr after exposure. *Cis*- and *trans*-permethrin were quantified using LC-MS/MS. Isotopically-labeled $^{13}\text{C}_6$ *trans*-permethrin served as a surrogate for sample recovery and $^{13}\text{C}_6$ *cis*-permethrin was used as internal standard post extraction to account for variance in LC-MS/MS analysis.

The modeling of *cis*- and *trans*-permethrin required attenuation of the *in vitro* hepatic clearance constant by a factor of 25%. This suggested the need to model the data with competitive inhibition. However, comparison of *in vitro* clearance rates for *cis*- and *trans*-permethrin individually and in the mixture did not suggest inhibition, as the rates were nominally the same. Furthermore, all time course concentrations (Figure. 3.5) were below estimated K_m (40 μM). These findings suggested that the need for attenuation is based on efficiencies of *in vitro* to *in vivo* scaling and can be accounted for through the parallelogram approach (Figure. 3.1).

The time-course of *cis*- and *trans*-permethrin in plasma after single, oral administration of 1 mg/kg permethrin (mixture of 40% *cis*, 60% *trans*) is shown in Figure. 3.5A and B. A similar profile in Figure. 3.5C and D is evident for the 10 mg/kg dosage and shows that *cis*-permethrin displays diffusion-limited kinetics in the brain. The mixture was modeled for both doses (Figure. 3.5E-H) and shows that the mixture resembles *cis*-permethrin, which is consistent with the more slowly cleared and transported compound influencing the characteristics of the mixture. Thus, the preliminary modeling of permethrin demonstrates that modeling of the mixture is simply the superposition of the *cis* and *trans* time-course profiles and is suited for dose metrics.

Figure 3.5: Modelling of *cis*- and *trans*-permethrin. Plots A, B 1 mg/kg permethrin p.o., and plots C, D are 10 mg/kg. Plots E-H are directly analogous to A-D, with simulation of mixture



As described earlier, there are three options of model structure for permethrin. These initial analyses suggest that for permethrin, both the lumped and individual diastereomer model of the mixture provide a reasonable approximation of the experimental data. The difference between the models is complexity on the one hand and parsimony on the other. In principle, the complex model describing each diastereomer allows better mapping to the biological system (“representativeness”). On the other hand, a simpler representation (i.e., more parsimonious) may be adequate as is the case when modeling a “richly perfused compartment” as opposed to simulating the individual organs that constitute this compartment. The parsimonious model for *cis*-permethrin serves the task of accounting for permethrin distribution although it may impose some imprecision.

The data suggest that there are no pharmacokinetic interactions between *cis* and *trans*-permethrin and that the brain concentrations of *cis*-permethrin are 2-3 times higher than *trans*-permethrin. In mice, *trans*-permethrin is more than 80 times less potent than *cis*-permethrin when injected directly into the brain (Lawrence and Casida, 1982). Because *cis*-permethrin dominates the brain concentrations and *trans*-permethrin is 80 times less toxic, it is likely that the *trans*-permethrin contributes less than 1% to the “total “permethrin” toxicity based on tissue concentrations. Thus the more parsimonious model would be one that described *cis*-permethrin alone, while still providing accurate estimates of the exposure to dose relationship. The example with permethrin indicates that focusing only on *cis*-permethrin and ignoring *trans*-permethrin would not significantly impact the uncertainty in the tissue concentration or risk estimate. One source of uncertainty in this analysis is that estimates of the relative potency of *cis*- and *trans*-permethrin come from *in vivo* data in mice and the model describes the pharmacokinetics in rats. *In vivo* data on the toxicity of *cis* and *trans*-permethrin are not available at this time in rats.

The balance between parsimony and accuracy is one that must be treated with caution. The decision to model a particular pyrethroid as either a total of its stereoisomers or as a mixture of stereoisomers depends on several factors. A first approach would be based on the availability of information on the differential toxicity of the stereoisomers. An inactive stereoisomer may not need to be included in the model if it does not alter the pharmacokinetics of the active isomers. It is likely that different stereoisomers will vary in their potency. In this case, there may be practical cut-offs for including a stereoisomer based on its relative potency and contribution to the toxic effect. For permethrin, it is clear that because the *trans* isomer likely contributes less than 1% to the toxic component found in the brain, simulation of the *trans* diastereomer does not significantly enhance our understanding of the risk estimate. For other chemicals it is likely that this practical cut-off will be less clear. Application of this approach to other pyrethroids, such as cypermethrin, is planned.

3.D. Extension of Rodent Models to Humans

3.D.1. Exposure

Exposure of the general population to pyrethroid insecticides occurs primarily from the dietary or from residential exposure (CDC, 2005). The National Report on Human Exposure to Environmental Chemicals of the Centers for Disease Control and Prevention provides an ongoing assessment of the U.S. population's exposure to environmental chemicals using biomonitoring. In 2001, urinary levels of pyrethroid metabolites were measured in a subsample of the U.S. National Health and Nutrition Examination Survey (NHANES) participants aged 6 years and older and were selected within specified age ranges to be a representative sample of the U.S. population (Table 3.3). The metabolites, FBPA (4-Fluoro-3-phenoxybenzoic acid urine) and *cis*-DBCA (*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid urine), specific to cyfluthrin and deltamethrin, respectively, were not detected for 95 percent of the sampled population. In contrast, *cis*-DCCA (*cis*-DCCA: *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid) and *trans*-DCCA (*trans*-DCCA: *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid), specific to permethrin, cypermethrin and cyfluthrin (see Figure. 3.5), were detected in the upper 75th, 90th and 95th percentiles. Since FBPA was not detected, it is concluded that these metabolites arise mainly from permethrin or cypermethrin, or both. Interestingly, at the higher percentiles, the most non-specific metabolite PBA (3-phenoxybenzoic acid) levels were approximately equal to the sum of *trans*-DCCA and *cis*-DCCA, further suggesting that cypermethrin or permethrin represent the main contribution. As described in the next section below, these urinary data can be used to test hypotheses regarding pathways of exposure to pyrethroids.

Table 3.3: Urine concentrations ($\mu\text{g/L}$ and $\mu\text{g/g}$ creatinine) of pyrethroid metabolites for the U.S. population aged 6-59 years, National Health and Nutrition Examination Survey, 2001-2002 (CDC, 2005)

	CC	LOD	GM	Percentiles				N
				50th	75th	90th	95th	
PBA		0.1	0.321	0.280	0.690	1.690	3.320	2539
	1	0.1	0.316	0.283	0.582	1.460	3.100	2538
FPBA		0.2	*	<LOD	<LOD	<LOD	<LOD	2539
	1	0.2	*	<LOD	<LOD	<LOD	<LOD	2538
<i>cis</i> -DCCA		0.1	*	<LOD	0.16	0.49	0.89	2539
	1	0.1	*	<LOD	0.219	0.436	0.78	2538
<i>trans</i> -DCCA		0.4	*	<LOD	0.41	1.2	2.5	2525
	1	0.4	*	<LOD	0.718	1.45	2.55	2524
<i>cis</i> -DBCA		0.1	*	<LOD	<LOD	<LOD	<LOD	2539
	1	0.1	*	<LOD	<LOD	<LOD	<LOD	2538

Values corrected for creatinine (CC) are denoted by 1, and are in units $\mu\text{g/g}$ creatinine; otherwise values are in units $\mu\text{g/ml}$ urine. LOD: Limit of detection. GM: Geometric mean N: Sample size. * Not computed; proportion of values below LOD was too large to provide a valid result. FPBA: 4-Fluoro-3-phenoxybenzoic acid urine. *cis*-DCCA: *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid urine. *trans*-DCCA: *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid urine. *cis*-DBCA: *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid urine. PBA: 3-Phenoxybenzoic acid urine

3.D.2. Use of human dose-excretion studies to develop human models

In a preliminary application the parallelogram approach (Figure. 3.1) a provisional model of permethrin disposition in humans was developed based on the Delta Model through a parallelogram approach (Figure. 3.1). Clearance of permethrin was estimated using pooled human hepatic microsomes (see Appendix B). A mixture of permethrin (40% *cis*, 60% *trans*) exhibited NADPH-dependent clearance of 122.5 ± 31.6 ml/min/kg BW and non-NADPH dependent clearance of 132 ± 18.1 ml/min/kg BW, suggesting predominant clearance by the esterase pathway in humans. The major products of hydrolysis, 3-PBA and DCCA were assumed to enter a central compartment and clear at a rate equal to $CL_{\text{met}}=K/V$, where $K=\ln(2)/t_{1/2}$ and V_{dss} is the volume of distribution at steady state. The half-life ($t_{1/2}$) was estimated at 13 hrs for each metabolite (Woollen et al., 1992). Using the methodology of Poulin and Theil (2002), $(V) = \sum (\text{tissue volume}) \times (\text{tissue:plasma}) + \text{plasma volume}$, V was estimated at 4.60 L/Kg for PBA and 3.971 L/Kg for DCCA.

The provisional model was then used to evaluate the contribution of pyrethroid residues in food. The levels of urinary metabolites of pyrethroids reported through the 2001-2002 U.S. National Health and Nutrition Examination Survey (NHANES) were used as a general estimate of population exposure to pyrethroids (Table 3.3). Dietary exposure to these pyrethroids was estimated using U.S. Department of Agriculture's Continuing Survey of Food Intakes by Individual (CSFII) and Pesticide Data Program (PDP)(USDA, 2005). Simulations

conducted with 75th, 90th, and 95th percentile of dietary intake accounted for only about 1%, 2%, and 2% of the respective percentiles of excreted DCVA reported in NHANES. This analysis suggests that food exposure may not be a major contributor of exposure. However, it is notable that refinements to the exposure assumptions and to the permethrin PBPK model are expected. Future studies will investigate both dietary and non-dietary exposure as a source of metabolites.

4. Evaluating Uncertainty and Variability in PBPK Models for Pyrethroids

Variability and uncertainty are concepts that have often been conflated in risk assessment considerations. More recently, it has been realized that it is important to consider the two concepts separately. Generally, variability refers to a characteristic of a population: variability is present when members of a population differ among themselves in some characteristics. This terminology is used whether the “population” is a collection of individuals (whether they be humans or rats), or even of identical experiments. It is possible to quantify variability, but not to reduce it by purely observational means. Uncertainty refers to the inability to ascertain the state of nature with certainty. The two concepts are often related. For example, measurements of two characteristics for a sample from a population, say height and weight in a sample of adult males from the US may be available. The regression coefficient relating weight to height in that sample would probably be positive, but would probably quantitatively differ in second and subsequent samples. The sample coefficients would *vary* among the samples, because the samples would vary among themselves. Each coefficient is an *estimate* of the linear relationship between height and weight in the full population. However, there is *uncertainty* about the true value, and the symptom of the uncertainty, in this case, is that estimates of the same quantity differ from each other. Thus, variability in a population gives rise to uncertainty about our uncertainty of population characteristics. Uncertainty of this sort can usually be reduced experimentally: in this case, by increasing the sample size or increasing the spread of heights in a sample.

This example allows another type of uncertainty that is often important in risk assessment to be considered, that of *model uncertainty*. In the example above, a linear relationship between height and weight was assumed. However, the true relationship may be more complex, and certainly depends upon other factors like age and health status. The presence of model uncertainty may affect the estimates of particular parameters, when alternative models contain the same parameter with the same meaning, and certainly affects the predictions made from the models.

Another commonality between variability and uncertainty is that it is typical, though not universal, to use the machinery of probability theory to model both concepts. Thus, we may approximate the variability of a characteristic in a population with a log normal distribution, and we may characterize our uncertainty about the value of a population parameter with a normal distribution. We may be justified in this latter

practice by the fact that the *variability* of estimates of the parameter may be characterized by a normal distribution. By extension, we may model uncertainty using probability distributions, even in the absence of a very plausible justification based on “long term frequency”. Bayesian methods do just this, and theory has shown the methodology to be internally consistent, and to give rise to rational support for decision making. It is important, though those analyses keep the two concepts in their proper places. For example, a statement about the internal dose of a pyrethroid in humans, given a particular exposure, may well be characterized in terms of the probability that the dose falls in particular ranges: say, 99.9% of the population would have a peak brain concentration $< x$ ng/l given oral exposure d mg/kg. This is a statement about variability. However, a number of sources of uncertainty are acting here, and we should add a statement of uncertainty, such as confidence limits: the 95% confidence limits on the 99.9 percentile of peak brain concentration is (w, y) ng/l.

Variability and uncertainty need to be considered in two separate venues and sets of inferences: the PBPK models as developed for rodents and the PBPK models as developed for humans and used for inferring internal dose in a risk assessment. Somewhat different issues arise in the two modeling efforts, so they are best treated separately. In particular, variability has somewhat different consequences in the two sets of models.

4.A. Rodent PBPK Models

The rodent models will be used as part of a parallelogram approach to support the process of extrapolating from *in vitro* data to develop an *in vivo* human PBPK model. An initial model, based on deltamethrin, was developed using both *in vitro* and *in vivo* data. Models for subsequent pyrethroids in rodents are expected to be developed from the deltamethrin model by replacing metabolic rate parameters, estimated *in vitro*, for those of deltamethrin. *In vivo* data for the subsequent pyrethroids are then used solely for testing the predictions of this new model. The point is to have no chemical-specific parameters in the model that are not estimable from *in vitro* data. To the extent this enterprise is successful; it is more plausible that models for human data should be taken as reasonable approximations to reality.

The following broad steps are required to achieve this end:

1. Estimate parameters for deltamethrin model, and iteratively improve the model until it adequately describes all *in vivo* data. At any step in this iterative process, priors for metabolic parameters are informative, derived from *in vitro* experiments (for example, if the estimated value for parameter μ_1 , the logarithm of metabolic rate parameter k_1 , is $\hat{\mu}_1$ with estimated standard error $\hat{\sigma}_{\mu_1}$, the prior density for the parameter, $p(\mu_1)$ is normal with mean $\hat{\mu}_1$ and standard deviation $\hat{\sigma}_{\mu_1}$), and similar informative priors for log partition coefficients, based on estimates of ratios of areas under the curve from pnd10 data (see description of their computation, above). Some sensitivity analysis needs to be carried out to see whether it is reasonable to treat the physiological parameters as fixed or to give

them informative priors, as well. Absorption and diffusivity coefficients are given diffuse priors. Finally, some parameters need to vary among individuals, to account for interindividual variability. In the example worked here, it is the absorption parameters that are allowed to vary, but, again, sensitivity analysis and comparing different parameterizations will tell where it is best to include interindividual variation. If \mathbf{Y} represents the *in vivo* time-course data, $\boldsymbol{\mu}$ the metabolic parameters, $\boldsymbol{\pi}$ the partition coefficients, $\boldsymbol{\varphi}$ the physiological parameters, \mathbf{M}_α the population mean and \mathbf{S}_α the population variance for the absorption parameters, $\boldsymbol{\alpha}$, $\boldsymbol{\delta}$ the diffusivity coefficients, and $\boldsymbol{\sigma}^2$ the error variance, then the posterior for

$$\boldsymbol{\theta} = [\boldsymbol{\mu}', \boldsymbol{\pi}', \boldsymbol{\varphi}', \boldsymbol{\alpha}', \boldsymbol{\delta}', \boldsymbol{\sigma}^2] \text{ is}$$

$$p_{\text{post}}(\boldsymbol{\theta} | \mathbf{Y}, \mathcal{M}) \propto p(\boldsymbol{\mu}) \times p(\boldsymbol{\pi}) \times p(\boldsymbol{\varphi}) \times p(\boldsymbol{\alpha} | \mathbf{M}_\alpha, \mathbf{S}_\alpha) \times p(\mathbf{M}_\alpha) \times p(\mathbf{S}_\alpha) \times p(\boldsymbol{\delta}) \times p(\boldsymbol{\sigma}^2) \times L(\mathbf{Y} | \boldsymbol{\theta}, \mathcal{M}), \quad (4.1)$$

where $p()$ represents the appropriate density function for the corresponding prior, \mathcal{M} a particular model, and $L()$ represents the probability of the data given the parameters and model. Markov-Chain Monte Carlo methods are typically used to generate samples from such a posterior distribution. The fit of each model \mathcal{M} to the data will be evaluated using posterior predictive distributions (see Gelman, et al. 2004, Chapter 6; Gelman, 2003), and compared among each other using the deviance information criterion (DIC).

2. Once an acceptable model for deltamethrin is settled upon, it remains to determine whether a new model, derived from the deltamethrin model by replacing only the metabolic parameters with estimates from appropriate *in vitro* data, and new estimates of the error variance, adequately predict new *in vivo* data for a second (and other) pyrethroids. The comparison will again be carried out using a procedure analogous to posterior predictive distributions, but this time, in place of a posterior distribution derived as for the deltamethrin model (that is, using the *in vivo* data), will be a distribution derived both from the posterior for the parameters of the deltamethrin model and new *in vitro* derived posteriors for the metabolic parameters, as well as estimates of error variances in the new dataset. Let $\boldsymbol{\theta}^*$ represent the parameter vector leaving out the metabolic parameters and variance parameters. What is needed is the conditional posterior distribution of $\boldsymbol{\theta}^*$ given $\boldsymbol{\mu}$ and $\boldsymbol{\sigma}^2$:

$$P_{\text{post}}(\boldsymbol{\theta}^* | \mathbf{Y}, \mathcal{M}, \boldsymbol{\mu}, \boldsymbol{\sigma}^2) \quad (4.2)$$

When multiplied by the posterior for the metabolic parameters and variances for the new chemical, the result is a distribution that can be used like a posterior distribution for comparing posterior predictions with the actual *in vivo* data. One approach for deriving (4.2) is to approximate (4.1) as a multivariate normal distribution. Then the conditional distribution in (4.2) is also multivariate normal, and its mean and covariance parameters are derivable from the mean and variance parameters of (4.1). Those are readily computed from the posterior samples. In the simulations to compare the posterior predictive distributions from this model to the *in vivo* data, first a sample

would be drawn from the new distribution of the metabolic parameters and the error variances, then a sample representing the remaining parameters would be drawn from the distribution (4.2), given those values in the first sample.

It is critical while evaluating the fit of this generic model to data for individual pyrethroids that the goal of the testing is to evaluate the ability of the model to predict relevant features of the data. So, for example, if peak blood level were to be taken as the most important concentration, it would be most important to evaluate the degree to which the model predicts peak blood level. The goal here should be goodness of fit of the model to the values that are important that the model reproduce, rather than evaluate the overall fit to the data.

4.B. Human PBPK Models

The human PBPK model would be used to convert modeled exposures into internal concentrations, to be used for predicting biological effects. There is little (though some) human data useful for evaluating the quality of the human model. Thus, the model's validity, while derived in part from comparisons with human data, is derived mostly exclusively from the validity of the *process* used to generate it. To the extent possible human *in vivo* data are compared to posterior using parameter distributions constructed as above for the rodent model. In addition to the uncertainty inherent in using these estimates, and, for some parameters, in the extrapolation of measured rodent values (e.g., partition coefficients) to humans, we need to consider variability of the human population for physiological parameters and, to the extent it can be evaluated, for metabolic parameters.

5. Summary

This issue paper provides a summary of key science issues EPA has encountered during the development of PBPK models for pyrethroids. The development of PBPK models for pyrethroids poses some challenges because of a number of uncertainties and data gaps. PBPK models are most useful when there is a known toxicophore, the model outputs (dose metric) are quantitatively linked to adverse effects, and there is data in both rodents and humans to assess model predictions of exposure/dose relationships. One major impediment to model development for the pyrethroids is their complex stereochemistry which complicates identification of the major toxicophores. Many of the pyrethroids on the market are composed of several stereoisomers. These stereoisomers vary in their neurotoxic effects and may have potential for pharmacokinetic interactions of the toxic stereoisomers. EPA has begun to develop both experimental and modeling approaches to address these issues. One initial proposal examines the utility of lumping all of the stereoisomers as one chemical. The “lumped” chemical would be modeled as one entity. Other alternatives for dealing with the stereoisomers in model development are also being considered. Also under consideration is the most appropriate dose metric. Currently, EPA has examined peak concentration of parent pyrethroid in blood and brain for bifenthrin and correlating this concentration to motor activity in rats. These dose metrics are promising, but others such as area under the curve, have not been examined or excluded. Another obstacle is the limited human data available on the pharmacokinetics of the pyrethroid pesticides. The EPA plans to take a family modeling approach in the development of PBPK models for pyrethroids. It is hoped that by taking this approach confidence in each of the models will increase as more chemicals are examined. The intention of EPA is to use PBPK models in assessing the potential health risks associated with exposure to these chemicals.

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