A pharmacokinetic model of *cis*- and *trans*-permethrin disposition in rats and humans with aggregate exposure application

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Abstract

Permethrin is a broad-spectrum pyrethroid insecticide and among the most widely used insecticides in homes and crops. Managing the risks for pesticides such as permethrin depends on the ability to consider diverse exposure scenarios and their relative risks. Physiologically-based pharmacokinetic (PBPK) models of deltamethrin disposition were modified to describe permethrin kinetics in the rat and human. Unlike formulated deltamethrin which consists of a single stereoisomer, permethrin is formulated as a blend of *cis*- and *trans*-diastereomers. We assessed time courses for *cis*-permethrin and *trans*-permethrin in several tissues (brain, blood, liver and fat) in the rat following oral administration of 1 and 10 mg/kg permethrin (*cis/trans* : 40/60). Accurate simulation of permethrin in the rat suggests that a generic model structure is promising for modeling pyrethroids. Human *in vitro* data and appropriate anatomical information were used to develop a provisional model of permethrin disposition with structures for managing oral, dermal, and inhalation routes of exposure. The human permethrin model was used to evaluate dietary and residential exposures in the U.S. population as estimated by EPA's Stochastic Human Exposure and Dose Simulation (SHEDS) model. Simulated *cis*- and *trans*-DCCA, metabolites of permethrin, were consistent with measured values in the National Health and Nutrition Examination Survey indicating that the model holds promise for assessing population exposures and quantifying dose metrics.

***Keywords**: pyrethroids; PBPK; aggregate exposure model*

Introduction

Exposure to pyrethroid insecticides has increased annually in number and as a percentage of all insecticide exposure incidents following the 2000-2005 phase out of organophosphates from residential uses (Power and Sudakind, 2007). Quantifying pyrethroid exposures to infants and children may be particularly important as experimental studies show there is substantial age dependence to their acute toxicity, in which neonatal rats are as much as an order of magnitude more sensitive than adults to pyrethroids (Sheets *et al*., 1994; Shafer *et al*., 2005; Tornero-Velez *et al.,* 2010; Kim *et al.*, 2010).

Although permethrin is one of many pyrethroids on the market, it is frequently detected in agricultural commodities (USDA, 2009), and in homes and child care centers (Heudorf and Angerer, 2001; Schettgen *et al.*, 2002; Tulve *et al*., 2006; Lu *et al*., 2006; Morgan *et al*., 2007; Tulve *et al*., 2008; Williams *et al*., 2008; Lu *et al*., 2009; Stout *et al.,* 2009; Tornero-Velez *et al.,* 2012), and its metabolites are detected in urine in the population (CDC, 2009). In the United States, permethrin is registered for use on numerous agricultural crops, livestock and livestock housing, modes of transportation, structures, public health mosquito abatement programs, and residential settings including use in outdoor and indoor spaces (U.S. EPA, 2006). About 70% of permethrin is used in non-agricultural settings (U.S. EPA, 2006). Lifestyle factors, including diet and the residential use of higher-exposure methods (spray cans, pest bombs, or sprays by professional

pesticide applicators) may increase exposure for segments of the population. Permethrin also has pharmaceutical uses as a pediculicide for the treatment of head lice, particularly with children (Tomalik-Scharte *et al.*, 2005; Naeher *et al.*, 2009).

Like the natural pyrethrins and other synthetic pyrethroids, permethrin exerts neurotoxicity in mammals and insects due to its interaction with sodium channels in the axons of the peripheral and central nervous systems (Soderlund *et al*., 2002; Soderlund, 2012). These molecular events manifest in behaviors which depend on structure; with Type I pyrethroids such as permethrin tending to evoke tremors and hyperactivity, and Type II pyrethroids (bearing an α-cyano moiety; e.g., deltamethrin) eliciting choreoathetosis and salivation (Crofton and Reiter, 1988; Soderlund *et al.*, 2002; Wolansky *et al*., 2006; Soderlund, 2012). Like other pyrethroids, permethrin is very lipophilic (Log P=6.1) yet highly susceptible to mammalian detoxification, thus it does not accumulate in the body (Elliot *et al*.,1973; Gaughan *et al*., 1976; Soderlund *et al*.,2002). However, despite its rapid clearance from the body, permethrin readily distributes to the nervous system (Gaughan *et al*., 1977, Anadon *et al*., 1991, Harrill *et al*., 2008). Commercial formulations of permethrin are a mixture of [1*RS*, *trans*] and [1*RS*, *cis*] stereoisomers, with the [1*RS*, *cis*] diastereomer pair having markedly slower clearance kinetics in mouse, rat, and human liver microsomes (Soderlund and Casida, 1977; Scollon *et al*., 2009).

Given the potential for human exposure to permethrin through the diet and its widespread use, we developed a pharmacokinetic (PK) model of permethrin in humans suited for assessing aggregate exposure via oral, dermal, and inhalation pathways. Due to the paucity of available human PK data, we used a physiologically-based PK (PBPK) approach to make use of *in vitro* and *in silico* information pertinent to permethrin pharmacokinetics (Andersen, 2003). We developed a rat model of permethrin disposition to evaluate this paradigm for extrapolation (*in vitro*-*in vivo*), and to better understand the sources of uncertainty that may impact a human model of permethrin disposition. We started model development with PBPK models of deltamethrin (Mirfazaelian *et al*., 2006; Godin *et al.*, 2010), a Type II pyrethroid and structural analog of permethrin, using *in vitro* clearance rates for permethrin. Herein we evaluate simulations of *cis*- and *trans*-permethrin in rat tissues and discuss the model accuracy. We developed a model of permethrin disposition in humans for assessing aggregate exposure to permethrin by different routes of exposure using clearance rates for human and information on the dose-excretion of various pyrethroids. Finally, we evaluate the ability of the human model to simulate urinary metabolites of *cis- and trans*-permethrin in the population.

Materials and Methods

Chemicals. All chemicals were analytical grade unless otherwise specified. *Cis*-permethrin (3-Phenoxybenzyl(1RS)-*cis*-3-(2,2-dichlorovinyl)-2,2-

dimethylcyclopropanecarboxylate, 99 % purity; "*cis*-permethrin") and *trans*-permethrin (3-Phenoxybenzyl(1RS)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 94 % purity; "*trans*-permethrin") were purchased from Chem Service (West Chester, PA). Technical grade permethrin (92% purity) used in dosing solutions consisted of a 40:60 blend of *cis*/*trans*, and was generously provided by FMC Corporation (Philadelphia, PA). Labeled *cis*-permethrin (phenoxy-$^{13}C_6$) and *trans*-permethrin (phenoxy-$^{13}C_6$) were purchased from Cambridge Isotope Laboratories (Andover, MA). Solvents, including acetone, hexanes (Fisher Scientific, Pittsburgh, PA) and methanol (VWR, West Chester, PA) were pesticide grade.

Animals, animal dosing and tissue extraction. Male 70 d old Long Evans rats were purchased from Charles River Laboratories (Raleigh, NC) and allowed to acclimate for a minimum of 4 days in an Association for the Assessment and Accreditation of Laboratory Animal Care approved facility. All animal procedures were approved by the Institutional Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory. Rats were housed in pairs in cages lined with heat-treated pine shavings bedding. Temperature, humidity and photoperiod were maintained at 21 ± 2°C, 50 ± 10% and 12 h light:12 h dark. Feed (Purina Rodent Chow 5001, Barnes Supply Co., Durham, NC) and tap water were provided *ad libitum*. The rats were dosed orally with 1 or 10 mg/kg permethrin in corn oil (1 mL/kg) and sacrificed at 1, 2, 3, 4, 6, 8, 12, 24, 36, or 48 hours. Four rats were sacrificed for each time point at each

dose level permethrin under CO_2-induced anesthesia. Cardiac blood was taken via heart puncture and the animals were exsanguinated. Post mortem, brain, abdominal subcutaneous fat, and liver tissues were collected. Whole blood was dispensed in 2 mL aliquots and frozen in a methanol/dry ice bath. The remaining tissues were flash frozen in liquid nitrogen, homogenized in a Spex CertiPrep 6850 freezer mill (Metuchen, NJ) and then stored at -80°C.

Blood samples for analysis were prepared from the 2 mL aliquots described above. The mass of fat, liver, and brain tissue used for analysis of each sample was targeted to be 300 mg for extraction of parent. Tissues were weighed and the mass recorded while the samples were still frozen. Prior to extraction, tissues were placed in culture tubes and spiked with $^{13}C_6$-*trans*-permethrin that served as a surrogate standard. Samples were extracted with a 3 X 5 mL acetone in hexane (1:4). The extracts were pooled and dried under a gentle stream of nitrogen. Sample extracts from blood and brain tissues were passed through silica Solid Phase Extraction (SPE) columns. Samples from fat and liver tissues were cleaned by Gel Permeation Chromatography. The eluents from both processes were dried under a stream of nitrogen, reconstituted in 1 mL methanol:water 9:1 and $^{13}C_6$-*cis*-permethrin added (internal standard). Analysis was performed using an Applied Biosystems API 4000 LC/MS/MS with a C-18 column (3 x 150 mm, 3.5 μM). The retention times of *cis*-permethrin and *trans*-permethrin were 3.9 min and 3.6 min, respectively. Positive ionization was used for all compounds and transition ion pairs and retention times were used to verify

the identities of analytes. The tissue concentration of parent pyrethroid was determined because the current hypothesis is that metabolism of pyrethroids is a detoxication mechanism (Soderlund *et al.,* 2002; Soderlund, 2012). The method limit of quantitation (LOQ) for *trans*-permethrin was 59 pg/ml blood, 1037pg/g brain, 1262 pg/g liver, and 351 pg/g fat. The LOQ for *cis*-permethrin was 294 pg/ml blood, 494 pg/g brain, 955 pg/g liver, and 583 pg/g fat. See Starr *et al.* (2012) for details on the tissue processing and analysis of extracts.

Pharmacokinetic analysis (Table 3-6). The rat tissue time course data was analyzed using PK Solutions v. 2 (Summit Research Services, Montrose, CO).

Model structure. A PBPK model of *cis*- and *trans*-permethrin was developed to account for their blood and brain availability, and thereby assess risk of acute toxicity in rats and humans. The structure was based on existing PBPK models of the Type II pyrethroid deltamethrin (Mirfazaelian *et al.*, 2006; Godin *et al.,* 2010; Tornero-Velez *et al.,* 2010). Mirfazaelian *et al.* (2006) modeled the disposition of deltamethrin in the adult male Sprague-Dawley rat using both flow-limited (brain, gastrointestinal tract, liver, and rapidly perfused tissues) and diffusion-limited (fat, blood/plasma, and slowly perfused tissues) rate equations. We maintained this structure with some modifications. We used first-order rate constants to describe oral absorption and fecal excretion, as described by Godin *et al.* (2010). Mirfazaelian *et al.* (2006) specified distinct red blood cell (RBC) and plasma compartments based on the observation that 93% of the

deltamethrin was associated with the plasma fraction. We modeled these as a single whole blood compartment since the deltamethrin concentration ratio (plasma:RBC) remained fixed over time. Both modifications were consistent with Godin *et al.* (2010) and Tornero-Velez *et al.* (2010), and as per the latter we maintained a flow-limited structure for the richly-perfused tissues (including liver), and modeled brain, fat, and slowly-perfused tissue with diffusion-limited kinetics.

Parameterization of the rat and human model. Anatomical parameters, blood flows and compartment volumes were taken from Brown *et al*. (1997) and summarized in Table 1. The gastric (0.01 h^{-1}) and intestinal (0.9 h^{-1}) absorption rate constants, and GI transfer rate constant (0.7 h^{-1}) were the same values used for deltamethrin by Mirfazaelian *et al*. (2006), and the same for both isomers. The fecal excretion constant (0.6 h^{-1}) was set to the value used by Godin *et al*. (2010), for both isomers. Tissue:blood partition coefficients (PCs) for liver, slowly-perfused and richly-perfused tissues were set to the PCs (tissue:plasma) used by Mirfazaelian *et al*. (2006). The fat tissue PC for deltamethrin (48.88) was nominal for *trans*-permethrin (50), but was increased 3-fold for *cis*-permethrin (150) to fit the data. The brain tissue PC for deltamethrin (0.22) was kept below unity for *trans*-permethrin (0.4) and above unity for *cis*-permethrin (1.5). The permeability coefficient for slowly-perfused tissue was set to the value used by Mirfazaelian *et al*. (2006) for both isomers. Permeability coefficients (specified as fractions of tissue blood flow) for fat and brain were fit by eye to 0.1 for fat, 0.003 for brain, for both isomers. Because of the absence of human

pharmacokinetic data, we maintained the same oral absorption rate constants, PCs, and permeability coefficients for rat and humans (Table 2).

To parameterize metabolic clearance in rat and human, we considered an *in vitro/in vivo* parallelogram approach where the clearance of *cis*- and *trans*-permethrin in rat liver microsomes would be scaled to parameterize the rat model, and adjustments needed to accommodate the fit would be applied accordingly in scaling the human model. However, uncertainties in the rat *in vitro* data complicated the selection of initial values. Clearance of *cis*- and *trans*-permethrin estimated from Michaelis-Menten constants (Vmax, Km) were plausible, but not those estimated by the half-life approach (<<Km); specifically, the expected pattern (clearance *trans* > clearance *cis)* was not observed (Fig.2). In contrast, human hepatic clearance rates determined by the half-life approach mirrored the finding of Soderlund and Casida (1977) who reported a 5-fold faster rate of clearance of *trans*-permethrin compared to *cis*-permethrin, in mouse microsomes. Scollon *et al*. (2009) observed a 12-fold faster clearance of *trans*-permethrin when *cis* and *trans* isomers were individually assessed, and a 7-fold difference if each was part of a typical 40:60 cis/trans blend (Fig.2). The rat data mirrored this pattern to a lesser extent and only when clearance was determined from the Michaelis-Menten constants (Vmax, Km); clearance of *cis*-permethrin was faster when determined by the half-life approach. To help resolve this disparity we optimized clearance rates based on the *in vivo* data. Fig. 2 shows the scaled rates compiled from Scollon *et al*. (2009). These rates were based on: (1) the

microsomal rates (ml/min per mg microsomal protein (MSP); (2) an MSP content of 52.5 and 45 mg/g liver for humans (Iwatsubo *et al*., 1997) and rats (Houston, 1994), respectively; and, (3) anatomical data in Table 1 (g liver per kg body weight). Clearance rates for *cis*- and *trans*-permethrin from rat blood were obtained from Crow *et al*. (2007) and set to zero in the human model as human serum does not contain sufficient esterases to metabolize pyrethroids (Crow *et al*., 2007). Clearance of *cis*- and *trans*-permethrin from intestinal tissue was based on rates from Nakamura *et al*. (2007), and a value of 2.1 MSP per g intestine was used for rat and human (Martignoni *et al*., 2006).

Initially, PCs and permeability coefficients for *cis*- and *trans*-permethrin were assumed to be equivalent to each other and equal to fitted values for deltamethrin (Mirfazaelian *et al*., 2006). The parameterization sequence was as follows: rat clearance rates were initially fit by eye conditional on the initial PC and permeability values; the aforementioned adjustments to PCs and permeability coefficients were made, and then rat clearance rates were optimized.

Aggregate model structure-human. The PBPK model was adapted to model human aggregate exposure to permethrin (Fig. 3). The following steps were taken: (1) a simple inhalation compartment was added; (2) a dermal compartment was added; and, (3) a one compartment pharmacokinetic model of metabolites *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid *(cis- and trans*-DCCA) was added.

The lungs were modeled as a well-stirred compartment with one-directional airflow in the region of gas exchange equal to alveolar ventilation rate Q_p, and with rapid equilibrium between lung air and lung blood. The concentration of permethrin in blood exiting the lungs, C_a, is described according to Ramsey and Andersen (1984) as

$$C_a = \frac{Q_P \times C_{inh} + Q_c \times C_{BLV}}{Q_c + Q_P \ / \ P_B}$$

Where C_{inh} is the inhaled concentration of permethrin, P_B is the blood:air partition coefficient, Q_c is the cardiac output, and C_{BLV} is concentration in the venous blood (see Appendix for details).

Dermal exposure was modeled as a one-directional diffusive process of permethrin across the stratum corneum (SC) and viable epidermis (VE). A two-compartment structure was employed with an empirical uptake rate (K_{ds}; h^{-1}) between surface and SC and an empirical uptake rate (K_{dve}; h^{-1}) between SC and VE. Separate sets of differential equations are used for *cis*-permethrin and *trans*-permethrin; although the rate constants are the same for both, this allows for different loading of each on the skin.

$$\frac{dAsurf}{dt} = -K_{ds} \times Asurf$$

$$\frac{dA_{SC}}{dt} = K_{ds} \times Asurf - K_{dv} \times A_{SC}$$

$$\frac{dA_{VE}}{dt} = K_{dVE} \times A_{SC} + Q_{SKN}\left(C_{art} - C_{VE}/P_{VE:B}\right)$$

where A_{surf} is the amount on surface, A_{SC} the amount in the SC, and A_{VE} the amount in the VE. The viable epidermis:blood partition coefficent is abbreviated as $P_{VE:B}$. Blood flow to the skin is abbreviated as Q_{SKN}. A portion of the slowly perfused compartment was allocated for the two-compartment skin model (Fig.3).

Single compartment model of* cis- *and* trans-*DCCA. We added a biomarker compartment to interpret human dose excretion studies for pyrethroids, and to fit uptake of permethrin by different routes (oral, dermal, and inhalation) in the model. The biomarker compartment was necessary to interpret permethrin biomarker data for the U.S. population, based on urine samples collected in the Center for Diseas Control National Health and Nutrition Examination Survey (NHANES) and tested as part of National Report on Human Exposure to Environmental Chemicals (CDC, 2009).

Neither permethrin nor its hydroxyl derivatives (Fig. 1a) are eliminated in urine (Gaughan *et al*., 1976). However, hydrolysis of *cis*- and *trans*-permethrin (Fig. 1c) renders phenoxybenzoic acid (3-PBA) and *cis- or trans*-DCCA (DCCA), each of which is excreted in urine. A mechanism proposed by Casida and Ruso (1980) shows that hydroxylation by cytochrome P450 (Fig. 1b) at the carbon proximal to the ester results in ester cleavage, rendering 3-PBA and DCCA.

We modeled *cis- and trans*-DCCA, as these are specific to permethrin, cypermethrin, and cyfluthrin, whereas 3-phenoxybenzoic acid (3-PBA) is common to a larger set of pyrethroids. A human dose-excretion study of oral and dermal exposure to cypermethin (Woollen *et al*.,1992) was employed to obtain the rate constants for urinary elimination of *cis/trans*-DCCA in humans from the plasma compartment. An elimination half-life of 12 h was estimated from urinary excretion of *cis/trans*-DCCA, and a rate constant of Kel= $\ln(2)/t_{1/2}$ = 0.06 was determined The study of Gaughan *et al*. (1976) on the fate of C14-labeled *cis*- and *trans*-permethrin in rat was used to estimate empirical stoichiometric coefficients relating metabolized *cis*- and *trans*-permethrin to urinary *cis*- and *trans*-DCCA, respectively; DCCAfrac=0.26 for *cis*-permethrin and DCCAfrac=0.73 for *trans*-permethrin. Details on the derivation of these coefficients are provided in the Supplemental section. The rate of production of DCCA to a central biomarker compartment (μmole/h) is given by:

$$\frac{dAhyd}{dt} = DCCAfrac \times (rLiv + rCaEP + rCaEGI) - Kel \times Ahyd$$

where *rLiv* is rate of metabolic hepatic clearance; rCaEP, the rate of metabolic blood clearance; and, rCaEGI, the rate of metabolic intestinal clearance. Separate equations were applied for *cis*- and *trans*-permethrin. The rate of urinary excretion (μmole/h) was computed with separate equations for *cis*- and *trans*-DCCA as:

$$\frac{dExcr}{dt} = Kel \times Ahyd$$

Rat model simulation and fit. All simulations were conducted using Matlab (Mathworks) using ode15s, a variable order method for solving stiff differential equations. Agreement between model predictions and experimental data was assessed with the goodness of fit approach of Krishnan *et al*. (1995). This index was used to compare experimental data and simulations. For a simulated time-course the index (I) is

$$I = \frac{\sqrt{\sum (data - prediction)^2}}{\sqrt{\sum data^2}}$$

where "data" represents the experimental data at various time-points, and "prediction" represents the simulated predictions at the same time-points. An overall index was calculated across compartments, by weighting according to the number of data points (N) in each compartment time-course:

$$I = I_A \left(N_A \Big/ N_A + N_B \right) + I_B \left(N_B \Big/ N_A + N_B \right)$$

where I_A and I_B are indices for two compartments A and B, and N_A and N_B are the corresponding number of data points in each compartment.

Intrinsic clearance for *cis*- and *trans*-permethrin in the rat was fitted by finding the optimal values for the function (I) for all compartments (blood, brain, liver, and fat) for the high 10 mg/kg dose. Optimization was performed with the Nelder-Mead

(1965) simplex algorithm, implemented in Matlab using the function *fminsearchbnd* (2012). This function is based on the Matlab function *fminsearch* allowing bound constraints for the optimized variables.

Simulation of oral and dermal exposures in humans. The model was used to interpret the case reported by Gotoh *et al*. (1998), which describes the clinical monitoring of an intentional poisoning from ingestion of permethrin. The 59 year old male (63 kg) was found unconscious alongside pools of vomit after imbibing a 600 ml solution of a 20% solution of permethrin (*cis/trans*: 43.5/56.5). The serum permethrin concentration was 214 ng/mL (*cis*/*trans*: 118/96) on admission, and increased to a maximum of 868 ng/mL (*cis*/*trans*: 615/253) 3-4 hours after ingestion. We evaluated the clearance of isomers for consistency (e.g., time to peak, the shape of the *cis* and *trans* time-courses, the relative persistence of *cis* and *trans* isomers) with the rat time courses and used the permethrin model to estimate the actual dosage.

The model was used to simulate excretion of total DCCA (*cis*/*trans* isomers not differentiated) following dermal application of a permethrin-based prescription cream for scabies (Tomalik-Scharte *et al*., 2005). In this study, 60 g of cream containing 5% permethrin (*cis*/*trans*: 25/75) for treatment of scabies was administered to the skin of the whole body of healthy volunteers. The simulation accounted for 12 hour application and removal. Urine was collected up to 168 h post-dose and analyzed for total DCCA. Individual cis and trans-DCCA were not

resolved therefore the same dermal rate constant (h^{-1}) was fit for *cis*- and *trans*-permethrin.

Simulation of urinary DCCA in NHANES samples

The goal of this simulation was to quantify dietary and residential exposure to permethrin and to compare model predictions with observed biomarker data. Accurate specification and parameterization of the uptake process (oral, dermal, inhalation) and the biomarker sub-model is critical to relate exposures to urinary metabolite levels. Probabilistic exposure modeling for dietary and residential permethrin exposure was conducted with the Stochastic Human Exposure and Dose Simulation Model for Multimedia, Multipathway Chemicals (SHEDS-Multimedia):Dietary Module and Residential Module (http://www.epa.gov/heasd/products/sheds_multimedia/sheds_mm.html; Zartarian *et al*., 2012; Xue *et al*., 2010). The SHEDS-PBPK biomarker simulation was conducted by processing the SHEDS output exposure profiles through the aggregate human permethrin model (Fig. 3). Residential and dietary exposures to permethrin were simulated for 8994 persons of 6 years age and older. For each person, hour-by-hour exposures were simulated for 21 days (504 hours). These 8994 persons represent the satisfactory merger within SHEDS of dietary and residential simulations, each consisting of 10000 persons. For the 6-12 year old group there were 978 persons simulated, 1025 persons for the13-19 year old group, 4407 persons for 20-49 year, and 2584 for the 50+ group. The aggregate exposure matrix served as input for the PBPK model. Each row of this matrix

provided an hourly update of the aggregate exposure vector, providing starting conditions to inform the initial state vector (ISV) for a one-hour PBPK simulation. Upon completion of the hour simulation, the model state variables provided information that was combined with information in the next row of the exposure vector to reset the ISV for the next hour of simulation. The dermal deposition and removal process (e.g., washing) were updated hourly by the SHEDS model. These processes were described with first-order rate constants (h^{-1}), thus it was possible for the SHEDS simulation to consider removal by dermal absorption prior to PBPK simulation. Studies evaluating the length of time to achieve pseudo steady-state conditions indicated that a 5-day simulation provided a reasonably stationary distribution of dose-metrics. Upon completion of a 6-day simulation, dose metrics pertaining to the individual were retrieved and stored. This was performed for all 8994 individuals, providing a distribution of dose-metrics for the population. Model evaluation was conducted by comparing the percentiles of the cumulative distribution function (CDF) of simulated *cis*- and *trans*-DCCA excreted urinary biomarker with the percentiles of the *cis*- and *trans*-DCCA reported in the '99-'00 and '01-02 survey years of the National Health and Nutrition Examination Survey (CDC, 2009).

Results

Disposition of* cis- *and* trans-*permethrin in the rat. The fate of *cis*- and *trans*-permethrin in the rat is summarized in Tables 3-6. Basic pharmacokinetic descriptors were computed directly from time-course data. The concentrations of

the *cis* isomer were always greater than those of the *trans* isomer of permethrin, ranging from 2- to 16-fold greater in blood and tissues. Peak blood and liver concentrations of *cis*- and *trans*-permethrin occurred at 1 h for both dose levels of administered permethrin (Table 3). The peak levels in fat and brain occurred at 1-12 h after administration. The fat had the highest and the liver the lowest maximum tissue concentrations for the two isomers at both dose levels (Table 3). The fat had the highest and the liver the lowest area under curve (AUC) for the two isomers at both dose levels (Table 4). For all tissues, the AUC for the *cis* isomer was 3- to 15-fold greater than that of the *trans* isomer at both dose levels. The tissue-blood ratios were highest in fat and lowest in liver (Table 5). In the fat, the ratios for the *cis* isomer were 1.5- to 2-fold greater than the ratios for the *trans* isomer. In brain and liver, the ratios were similar except for the 10 mg/kg dose in the brain, where the *cis* isomer ratio was about 3-fold greater than the *trans* isomer ratio. The elimination half-life of *cis*- and *trans*-permethrin was rapid in blood and liver (< 1 h), slightly greater in brain (1-5 h, and greatest in the fat (5-56 h). There was a trend of a larger half-life for the *cis* isomer at both dose levels.

Individual blood and brain concentrations of *cis*- and *trans*-permethrin were normalized by dose administered (1 and 10 mg/kg). The scaled time courses for blood concentrations (Fig. 4) overlap within 95% mean confidence intervals, indicating that the clearance kinetics of permethrin are first-order in this dose regime. Scaled time courses for *cis*-permethrin concentrations in brain also overlapped with exceptions at 2 and 48 h. Concentrations of *trans*-permethrin in

brain also showed the apparent outlier at 2 h. The findings are consistent with non-saturable kinetics for deltamethrin following oral administration of 2 and 10 mg/kg (Mirfazaelian *et al.,* 2006).

Rodent Model

Simulations of *cis*- and *trans*-permethrin are shown in Figure 5. The data were more variable at the 1 mg/kg dose (lower) yet maintained the same profiles for each compartment as with the higher 10 mg/kg dose. The intrinsic clearance of *cis*-and *trans*-permethrin were therefore optimized using the higher dose data. The optimized value for *cis*-permethrin clearance was consistent regardless of whether blood, brain, liver, and fat were individually excluded from the optimization, giving a value of 6.2, 6.3, 5.9 and 6.2 L/h per kg, respectively. The nominal value for clearance of cis-permethrin (6.2 L/h/kg) was the same regardless of whether the starting value for optimization was 1, 10, or 100 L/h/kg. The optimized value was near the (Vmax/Km)-based determination of 7.0 L/h/kg, when *cis*-permethrin was part of a 40/60 *cis/trans* blend (Fig. 2).

We optimized the clearance rate for *trans*-permethrin using blood, brain, and fat data, yet excluding the liver data. Fitting the liver data worsened the fit with other compartments. We speculate this was because of the larger proportion of liver data below the LOQ. With liver data included, hepatic clearance (95-102 L/h/kg) was considerably outside the *in vitro* range of 17-33 L/h/kg for *trans*-permethrin (Fig.2), for the individual isomer data and 40:60 blend data, and for both methods

(the half-life approach and Vmax/Km approach). When liver data was excluded the mean clearance for *trans*-permethrin was 24.5 L/h/kg regardless of the optimization starting point, and agreed with the *in vitro* estimates *trans* clearance (Fig.2). The model fit gave an overall discrepancy index of 0.47 for *cis*-permethrin and 1.24 for *trans*-permethrin. Fits were better for *cis*- and *trans*-permethrin in the fat at both doses suggesting that the body burden for this lipophilic compound is well described over time. Based on these fits and mass balance calculations, we estimate an oral absorption of 66% of the administered dose of permethrin.

Human Model

The rat optimization indicated faster clearance for *trans*-permethrin (3.5-fold faster than *cis*-permethrin). Human clearance rates, based on the half-life approach, indicated a 7-fold difference (Fig.2). We therefore used these *in vitro* rates without adjustments in the human model; 13.6 L/h/kg and 1.9 L/h/kg, respectively, for *trans*- and cis-permethrin. Using human anatomical data (Table 1) and human-specific *in vitro* data (Table 2), we parameterized, a human model of permethrin disposition. Studies on the toxicokinetics of permethrin following human exposure were not available aside from a case report of an apparent intentional poisoning from ingestion of permethrin (Gotoh *et al*., 1998). Although the exposure was not controlled, consistency with the rodent pharmacokinetics was evident. The serum concentration of *cis*- and *trans*-permethrin peaked 3-4 hours after ingestion and then rapidly declined. Although the victim imbibed a

lower proportion of the *cis* isomer (43.5/56.5 cis/trans), the serum concentration of *cis*-permethrin exceeded *trans*-permethrin, which dropped below detectable levels within 25 hours. This clinical observation indicated that the differential residence time of *cis* and *trans* isomer is consistent with rat pharmacokinetics. Based on a matching profile for *cis*-permethrin (Fig. 6), we estimate that the patient absorbed a dose of 630 mg permethrin; however, this is merely a point estimate given the uncertain timing of events. The main finding we wish to highlight is relative levels of *cis*- and *trans*-permethrin blood.

Because humans are exposed to permethrin by several routes (oral, dermal and inhalation), the model was adapted to consider these exposure routes (Fig. 3). The model is consistent with the findings of 'human dose-excretion' studies for pyrethroids which relate exposure to excreted metabolites, but do not evaluate tissue-distribution (Table 7). Urinary metabolites accounted for 35-60% of the oral dose of permethrin or cypermethrin. In contrast, urinary metabolites accounted for 0.1-1.2% of the applied dermal dose of these pyrethroids. To estimate a dermal absorption for humans we modeled the data of Tomalik-Scharte *et al*. (2005) who examined dermal excretion of DCCA following a 12 hour topical application of a prescription pediculicide cream containing 5% permethrin. Figure 7 shows excretion kinetics for 6 healthy subjects following topical application of the cream. Peak excretion rates occurred in about 1 day following exposure. Absorption rate constants, describing transfer of permethrin from the skin surface to stratum corneum, ranged from 0.001-0.0025 h^{-1} to

successfully model the peak and subsequent excretion out to 44 h. Excretion was under-predicted for 2 of 6 subjects at 92 h and 3 of 6 at 164 h.

Figures 8 and 9 provide comparison of the simulated urine concentrations of *cis*- and *trans*-DCCA with measured values from NHANES samples (CDC, 2009). In both figures, the upper region provides comparisons for entire age range, and towards the bottom, comparisons for children, teens, and adults, respectively. Comparisons are provided for the 75^{th}, 90^{th}, and 95^{th} percentile of each distribution. The NHANES data includes a line though the percentile denoting a 95% confidence interval. The general agreement between simulation and measured values supports use of the coupled SHEDS-PBPK models to assess population exposure to permethrin, and supports parameterization of the PBPK uptake processes. Higher exposure among the 6-12 age group is attributed to greater hand-to-mouth behavior, which is even greater at younger ages (Zartarian *et al*., 2012). The 95% confidence intervals (CI) on the simulated percentiles are based on sample theory. These CI relate to the uncertainty of projecting from a finite number of simulated individuals to a population of infinite size and assume that parameters are measured without error. As such, these CI underestimate the true uncertainty introduced by parameter uncertainty.

Discussion

In their work examining the relationship between pyrethroid structure and rates of hydrolysis and oxidation by mouse liver microsomal enzymes, Soderlund and Casida (1977) report hydrolysis rates which are different for *cis*/*trans* diastereomer pairs yet similar among enantiomer pairs. This finding motivated us to focus on *cis*-permethrin and *trans*-permethrin kinetic differences in the PBPK model. We also note that *cis*- and *trans*-permethrin are often differentiated in environmental surveys and exposure studies, whereas enantiomer pairs are not often resolved. The model structure borrows from Mirfazaelian *et al*. (2006) and Godin *et al*. (2010), the latter having hypothesized diffusion limitation for all tissues. We modeled the liver as a flow-limited compartment because *trans*-permethrin was so rapidly cleared from the liver so as not to suggest a full diffusion-limited structure.

The permethrin model captured important aspects of permethrin kinetics that are consistent with deltamethrin. This suggests that a common model structure is feasible for modeling pyrethroids. In rats, the brain:plasma distribution ratio of deltamethrin is estimated at 0.22 (Mirfazaelian *et al*., 2006). A comparable brain:blood ratio of 0.4 was observed for *trans*-permethrin. A value above unity, 1.5, was required to account for peak *cis*-permethrin in brain. This is consistent with Anadon *et al*., (1991) who reported brain:plasma distribution ratios (for total permethrin based on 48-h AUCtissue: AUCplasma) of 1.16 (cerebellum) to 4.27 (frontal cortex). The authors report a liver:plasma ratio of 0.44, consistent with

our finding. This agreement is in spite of the large oral dose administered of 460 mg/kg administered in their study. Anadon *et al*. (1991) report half-lives for brain regions of 13.7-23.1 hours and a plasma half-life of 12.4 hour, longer than half-lives for brain (4.7-4.9 h) and blood (1 h) observed in this study, suggesting saturation of metabolism at the ~0.5 g/Kg doseage. Even though the administered dose in our study had a greater proportion of the *trans* isomer, blood and tissue concentrations of *cis*-permethrin were greater than *trans*-permethrin. This is consistent with marked lability of the ester linkage for the *trans* configuration.of permethrin. The highest concentration of permethrin was in the fat (based on AUC), and this owes to the lipophilicity of permethrin. The next highest levels were in the brain, but were not high enough to achieve physical signs of neurotoxicity in the animals.

The liver is the main site of metabolic clearance of pyrethroids in rats and humans. Ross *et al*. (2006) has shown that the rat carboxylesterases, Hydrolases A and B, which are the most abundant rat hepatic carboxylesterases (Morgan *et al*., 1994), have a higher affinity and turnover numbers for *trans*-permethrin than *cis*-permethrin. Similarly, human carboxylesterase-1 and -2 cleave the ester linkage of the *trans* isomer more efficiently than the *cis* isomer of permethrin (Ross *et al*. 2006). This difference in metabolism of *cis*- and *trans*-permethrin explains the difference in their tissue levels. The *in vitro* data of Scollon *et al*. (2009) corroborates this finding for humans (clearance of *trans* > *cis)*, yet is ambiguous for the rat. Clearance estimates based on the ratio of

Michaelis-Menten constants (Vmax, Km) support the finding of the clearance *trans* > clearance *cis*. In contrast, their implementation of the first-order approach (Obach *et al*., 1997) indicated slightly greater clearance rates for *cis* (Fig. 2). Optimization of the intrinsic clearance rates supported the "*trans* >*cis"* case. Indeed, for a wide set of pyrethroids, clearance of the *trans* isomer is faster than *cis* isomer, and this owes to the stereoselectivity (preference for *trans*) of the catalytic triad and surrounding region within the esterase, a feature which is conserved across species (Buchwald and Bodor, 1999; Chang *et al*., *in press;* Hosokawa, 2008).

We recognize that we were not able to describe rapid clearance of *trans*-permethrin in the liver. Cytosolic and microsomal fractions of rat and human liver metabolize permethrin (Choi *et al*., 2002; Ross *et al*., 2006; Crow *et al*., 2007; Nakamura *et al*., 2007; Scollon *et al*., 2009). Approximately 40% of the esterase metabolism of *trans*-permethrin in human and rat liver is by the cytosolic esterases. Increasing the *in vitro* estimates of *trans*-permethrin clearance (17-33 L/h/kg BW) by 40% gives a range (23.8-46.2 L/h/kg BW) which includes the optimized value (24.5 L/h/kg BW) yet is still well below optimizations which included the liver (95-102 L/h/kg BW). *Cis*-permethrin is cleared entirely by cytochome P450 (Scollon *et al*., 2009), which is membrane bound, thus a similar adjustment would not apply.

Simulation of the accidental poisoning described by Gotoh *et al.* (1998) and the dermal exposure study of Tomalik-Scharte *et al*. (2005) indicate that the model captures basic features of permethrin pharmacokinetics in humans. Some of these features are invariant. Higher tissue levels of *cis*-permethrin relative to *trans*-permethrin are expected for humans exposed to permethrin (often a 40:60 *cis*/*trans* blend). This pattern was observed in the blood of the 59 year-old who ingested permethrin (Fig 6). However, variation in human physiology will influence pharmacokinetics and dose metrics. We observed a modest variability in the dermal rate constant (0.001-0.0025 h^{-1}) in fitting the dermal study of Tomalik-Scharte *et al*. (2005). Population variation in oral uptake is also expected. Sensitivity analysis conducted by Mirfazaelian *et al*. (2006) indicates that metabolic rate constants (Vmax, Km) and brain:plasma PC are an important determinant of peak brain deltamethrin. Age-dependence in the oxidative and hydrolytic rate constants for deltamethrin metabolism liver among post natal day 10, 21, 40, and 90 day old rats brought about a 4-fold difference in the 24 h brain deltamethrin AUC (Tornero-Velez *et al*., 2010). Specific enzymes can be targeted in studies of human variance. *In vitro* studies by the parent depletion approach suggest that rat cytochrome P450 isoform 2C11 and human isoform 2C19 would be most active in the liver towards permethrin (Scollon *et al*., 2009). The benefit of PBPK modeling is the ability to incorporate species- and subject-specific information.

The SHEDS-PBPK simulation of population exposure to permethrin provided estimates of *cis*-DCCA and *trans*-DCCA metabolites in urine in approximate agreement with sampled values. We estimate three-fold greater urinary excretion of *trans*-DCCA compared to *cis*-DCCA. This result is within the range observed for the trans-DCCA/*cis*-DCCA ratio for the population surveyed in the1999-2000 National Health and Nutrition Examination Survey (1.43 (10^{th} percentile) -4.44 (90^{th} percentile), N=1952; CDC, 2009). While this exercise does not validate the PBPK model, it supports the SHEDS-PBPK modeling paradigm and supports the specification of uptake processes in the aggregate PBPK model. Due to 24-h integration over time, urinary dose metrics (DCCA concentrations) are damped relative to peak brain levels, lessening the impact of some parameters but increasing the impact of others (e.g., variance in urine flow, dermal uptake). Compensating errors may mask agreement of predicted and empirical biomarkers, necessitating evaluation of individual uptake processes (dermal/oral/inhalation) when feasible. Additional work is needed to investigate the contribution of cypermethrin and cyfluthrin at different percentiles of the *cis* and *trans*-DCCA distributions. The national survey of pesticide residues in homes (Tulve *et al*., 2006) reported higher detection frequencies for *cis*-permethrin (72%) and *trans*-permethrin (72%), compared with cypermethrin (23%) and cyfluthrin (7%). However, the mean cypermethrin surface loading (0.26 ng/cm^2, SE=0.09, N=168) was higher than *cis*-permethrin (0.16 ng/cm^2, SE=0.04, N=167) and similar to *trans*-permethrin (0.3 ng/cm^2, SE=0.09, N=167). A lower mean surface loading was reported for cyfluthrin (0.13 ng/cm^2, SE=0.07, N=168).

At the 75th percentile, all cypermethrin values were below the detection limit, thus any contribution by cypermethrin will likely occur at the upper percentiles. In a national survey of pesticide residues in 500 homes, Stout *et al*. (2009) reported higher detection frequencies for *cis*-permethrin (89%) and *trans*-permethrin (89%), compared with cypermethrin (46%) and cyfluthrin (17%), yet higher mean loadings for cypermethrin (2.9 ng/cm^2; SE=0.86; N=480) compared with *cis*-permethrin (1.4 ng/cm^2, SE=0.22, N=459) and *trans*-permethrin (2.2 ng/cm^2, SE=0.37, N=448). The 75th percentiles for cypermethrin, *cis*-permethrin, and *trans*-permethrin were 0.16, 0.59, and 0.74ng/cm^2, respectively, indicating that a contribution from cypermethrin will likely occur at the upper percentiles. A mean loading for cyfluthrin was not computed, but the 95th percentile loading (0.5 ng/cm^2) was considerably lower than *cis*-permethrin (6.7 ng/cm^2), *trans*-permethrin (10.1 ng/cm^2) and cypermethrin (10.4 ng/cm^2). These finding indicate that non-dietary exposure to cypermethrin will contribute to urinary *cis*- and *trans*-DCCA at the upper percentiles, and cyfluthrin is expected to have only a very minor role. Any discrepancies between simulated and empirical urinary DCCA are grounds to motivate a reevaluation of assumptions in both models, SHEDS and PBPK, including exposure assumptions, kinetic constants, and physiological parameters.

Conclusion

We describe a PBPK model for assessing aggregate exposures to permethrin. The description of pharmacokinetics in humans is informed by the properties of

permethrin, PBPK models of deltamethrin in rat, and permethrin *in vitro* clearance data. The model is adapted with a biomarker sub-model to evaluate exposure estimation in probabilistic risk assessment applications. NHANES has the largest database of population urine DCCA measurements; however, the lack of accompanying exposure data (CDC, 2009) indicate that care must be taken in interpreting results. What we present is preliminary: (1) we intend to use a Bayesian-calibrated PBPK model in future evaluations to account model parameterization uncertainty; (2) variability sampling was not fully conducted (we considered variability in body weight as informed by SHEDS, but did not consider other forms of pharmacokinetic variance such as age-dependent clearance); and, (3) uncertainty sampling of the SHEDS parameter space was not conducted.

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FIGURE LEGENDS

Figure 1**.** Metabolic clearance of permethrin [1RS,3RS:1RS,3RS(*cis:trans*)], cypermethrin [*RS*- α-cyano;1*RS*,3*RS*;1*RS*,3*SR*(*cis:trans*)] or deltamethrin-[1R; *cis*; alpha S]. Oxidation via cytochrome P450 yields hydroxyl derivatives (**a.**) of the parent compound (e.g., 4'-hydroxy permethrin). Hydrolysis (**c.**), via carboxylesterases, yields a dichlorovinyl acid (permethrin, cypermethrin) or dibromovinyl acid (deltamethrin) and phenoxybenzyl alcohol. An important pathway for urinary metabolite of *cis* isomers (Casida and Ruso, 1980), oxidation leads to ester cleavage products following hydroxylation at the carbon proximal to the ester (**b.**).

Figure 2. Clearance of *cis*- and *trans*-permethrin in rat and human microsomal, determined from individual isomers and as part of 40:60 *cis/trans* blend. Clearance rates were determined by the half-life approach ([substrate] <<Km) or the Michaelis-Menten constants (Vmax/Km). Data of Scollon *et al*. (2009). Calculations are available in the Supplemental section.

Figure 3. PBPK model of permethrin for assessing aggregate exposure (via oral, dermal, inhalation routes). Delivery of permethrin to the gastrointestinal tract, liver and rapidly-perfused tissues is flow-limited, while delivery to the remaining

tissues (brain, fat and slowly-perfused tissues) is diffusion-limited. See Supplemental section for model equations.

Figure 4. Blood and brain concentrations of *cis*- and *trans*-permethrin for rats given oral doses of 1 and 10 mg/kg of permethrin. The blood and brain concentration time courses (scaled by dose) are shown in the semi-log plots for 1 to 48 h. The points represent the means (N=4) of the individual measurements for a given time and dose, while the error bars represent the 95% confidence intervals for the means.

Figure 5. Model-simulated *cis*- and *trans*-permethrin in rats, and observed concentrations (○, *cis*-permethrin; ◊, *trans*-permethrin) in the blood, brain, liver, and fat following an oral dose of either 1 or 10 mg/kg permethrin.

Figure 6. Simulation of *cis*-permethrin (—) and *trans*-permethrin (--) in human blood (data of Gotoh *et al*., 1998) following an oral dose of 10 mg/kg permethrin (*cis*:*trans*: 43.5:56.5).

Figure 7. Simulation of the rate of excretion of hydrolyzed products of permethrin following its topical administration in humans. Data of Tomalik-Scharte et al. (2005).

Figure 8. SHEDS-PBPK simulated dietary and residential exposures to permethrin in the population: comparison of simulated and NHANES sampled *cis*-DCCA. A total of 978 persons were simulated for 6-12 year olds, 1025 persons for 13-19 year olds, 4407 persons for 20-49 year olds, and 2584 persons for the 50 and above age group.

Figure 9. SHEDS-PBPK simulated dietary and residential exposures to permethrin in the population: comparison of simulated and NHANES sampled *trans*-DCCA. A total of 978 persons were simulated for 6-12 year olds, 1025 persons for 13-19 year olds, 4407 persons for 20-49 year olds, and 2584 persons for the 50 and above age group.

Table 1. Physiological parameters for use in rat and human PM PBPK models

	rat	human
	mean	**mean**
Parameter		
BW (Kg)	0.295	70
Cardiac Flow (QC, L/h/kg$^{0.75}$)	14.1	15.3
Tissue Volume (% body weight)		
Blood	7.4	7.9
Brain	0.6	2
Liver	3.4	2.6
GI tract	2.7	1.7
Skin	19	3.7
Rapidly Perfused	1.5	1.7
Poorly Perfused	59.4	43.7
Fat	7	21.4
Tissue Blood Flow (% cardiac output)		
Brain	2	12
Liver (Total)		
Portal (GI tract)	15.1	19
Arterial	2.4	6
Skin	5.8	5
Rapidly Perfused	21.1	25.5
Poorly Perfused	52.4	32.5
Fat	7	5
Blood Volume Fraction (% of Tissue)		
Brain	3	4
Fat	2	2
Poorly Perfused Tissue (muscle)	4	1

Values from Brown et al. (1997). Rapidly-perfused tissue value was computed as sum of heart, kidneys, and lungs. Poorly perfused tissue determined as BW- volume of excluded tissues (bone, GI contents, and non perfused tissues)- volume of rapidly-perfused tissues

Table 2 Permethrin-specific PBPK model parameters

Parameter	Rat Models			Proposed Human Models	
	deltamethrin	*cis*-PM	*trans*-PM	*cis*-PM	*Trans*-PM
Partition Coefficients					
Liver:blood (P_L)	0.44	0.44	0.44	0.44	0.44
Fat:blood (P_F)	48.88	150	50	150	50
Brain:blood (P_{BRN})	0.22	1.5	0.4	1.5	0.4
Slowly Perfused Tissue: blood (P_S)	5.59	5.59	5.59	5.59	5.59
Richly Perfused Tissue: blood (P_R)	0.44	0.44	0.44	0.44	0.44
Permeability Coefficients (fraction of tissue flow (L/h))					
Fat (PAFC)	0.016	0.1	0.1	0.1	0.1
Brain (PABRNC)	n/a	0.003	0.003	0.003	0.003
Slowly Perfused Tissue (PASC)	0.7	0.7	0.7	0.7	0.7
Rate Constants					
Stomach-intestine Transfer (Ksi) (hr^{-1})	0.7	0.7	0.7	0.7	0.7
Stomach Uptake (Ks) (hr^{-1})	0.01	0.01	0.01	0.01	0.01
Intestinal Transfer (Ki) (hr^{-1})	0.9	0.9	0.9	0.9	0.9
Fecal excretion (Kfec) (hr^{-1})	n/a[b]	0.59	0.59	0.59	0.59
Intestinal Clearance (CL_{ESTGI}) (L/h)	n.s	0.04	0.4	0.0	0.78
Blood CaE (L/h/kg)	0.06	0.08	0.31	n.s.	n.s.
Liver Clearance (L/h/kg)	1.69	6.2[c]	24.3[c]	1.86[d]	13.6[d]

n.s., not significant; n/a, not applicable, [a] Model of Mirfazaelian et al 2006,
[b] Mirfazaelian et al 2006 proposed a nonlinear clearance mechanism of fecal excretion
[c] optimized
[d] in vitro; based on incubation of human liver microsomes with 40:60 cis/trans blend (Fig. 2)

Table 3 Maximum tissue concentration/time of *cis*- and *trans*-PM in tissues of rats following oral administration of permethrin (40:60, *cis:trans*).

Dose	Brain		Blood		Fat		Liver	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
1 mg/kg	89[a]/2[b]	43/2	41/1	15/1	350/2	186/2	16/1	1/1
10 mg/kg	160/3	22/1	342/1	123/1	1412/12	456/4	129/1	15/1

[a]ng/g for tissue and ng/ml for blood

[b]h

Table 4 Area under of the curve for *cis*- and *trans*-PM in blood and tissues of rats following oral administration of permethrin (40:60, *cis:trans*).

Dose	Brain		Blood		Fat		Liver	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
1 mg/kg	303[a]	113	58	23	7319	1332	37	12
10 mg/kg	1426	97	663	160	57472	8874	308	29

[a]ng/g-h, from time 0 to 48 h

Table 5 Empirical distribution ratios (AUCtissue:AUCblood) of *cis*- and *trans*-PM following oral administration of permethrin (40:60, *cis:trans*) to rats.

Dose	Brain		Fat		Liver	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*
1 mg/kg	5.2	4.9	126.2	57.9	0.6	0.5
10 mg/kg	2.2/[a] 1.5	0.6/ 0.4	86.7/ 150	55.5/ 50	0.5/ 0.44	0.2/ 0.44

[a] Tissue: blood partition coefficients (Table 2)

Table 6 Elimination half-lifes (h) of *cis*- and *trans*-PM from blood and tissues of rats following oral administration of PM (40:60, *cis:trans*).

Dose	Brain		Blood		Fat		Liver	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
1 mg/kg	4.9	0.8	1.0	0.5	56.3	5.2	1.1	0.9
10 mg/kg	4.7	2.4	1.0	0.6	84	10.6	1.3	0.6

Table 7 Human dose-excretion studies with pyrethroids

Pyrethroid	Route	N	Concentration	Eliminated in Urine (% Dose)	$t_{1/2}$ (h)	trans-:cis-DCCA	Source
permethrin	oral	[a]	2.7 and 4.2 mg	35%	nd	nd	Bartlett and Hubbell, 1987[b]
cypermethrin	oral	6	3.3 mg; subjects: 61-80 kg	36%	13	2	Woollen et al., 1992
cypermethrin	oral	4	0.25 - 1. 5 mg in 0.2 ml cornoil	78.0%	nd	1.6	Eadsforth and Baldwin., 1983
alpha-cypermethrin	oral	6	0.25-0.75 mg in 0.2 ml cornoil	43.0%	24	nd	Eadsforth et al., 1988
pyrethrin	oral	3	3.3-5.4 µg/kg in 50:50 ethanol:water	60.0%	4.2	nd	Leng et al., 2006
permethrin	dermal	[a]	2.7 and 4.2 mg	1.2%	nd	nd	Bartlett and Hubbell, 1987 [b]
permethrin	dermal	6	5% prescription cream	0.47%	28.8	nd	Tomalik-Scharte et al., 2005
cypermethrin	dermal	2	25 mg/ 50 cm^2	0.10%	nd	nd	Eadsforth et al., 1988
cypermethrin	dermal	6	31 mg/ 800$cm^{2;}$ subjects: 62-82 kg	1.2%	16.5	1.2	Woollen et al., 1992
pyrethrin	dermal	6	0.3% prescription cream	body: 1.9% scalp: 7.5%	50	nd	Wester et. al., 1994
cyfluthrin	aerosol inhalation	9	40 and 160 mg/$m^{3;}$ subjects: 51-91.3 kg	51.45%	*cis:* 6.9; *trans:* 6.2	1.9	Leng et al., 1997

[a] Unknown N; [b]Report from Burroughs Welcome Co., Bartlett and Hubbell, 1987; nd= not determined,