Tissue time course and bioavailability of the pyrethroid insecticide bifenthrin in the Long-Evans rat

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Abstract
1. Pyrethroids are neurotoxic and parent pyrethroid appears to be toxic entity. This study evaluated the oral disposition and bioavailability of bifenthrin in the adult male Long-Evans rat.

2. In the disposition study, rats were administered bifenthrin (0.3 or 3 mg/kg) by oral gavage and serially sacrificed (0.25 h to 21 days). Blood, liver, brain and adipose tissue were removed. In the bioavailability study, blood was collected serially from jugular vein cannulated rats (0.25 to 24 h) following oral (0.3 or 3 mg/kg) or intravenous (0.3 mg/kg) administration of bifenthrin. Tissues were extracted and analyzed for bifenthrin by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

3. Bifenthrin concentration in blood and liver peaked 1–2 h postoral administration and were approximately 90 ng/ml (or g) and 1000 ng/ml (or g) for both tissues at 0.3 and 3 mg/kg, respectively. Bifenthrin was rapidly cleared from both blood and liver. Brain concentrations peaked at 4–6 h and were lower than in blood at both doses (12 and 143 ng/g). Bifenthrin in adipose tissue peaked at the collected time points of 8 (157 ng/g) and 24 (1145 ng/g) h for the 0.3 and 3 mg/kg doses, respectively and was retained 21 days postoral administration. Following intravenous administration, the blood bifenthrin concentration decreased bi-exponentially, with a distribution half-life of 0.2 h and an elimination half-life of 8 h. Bifenthrin bioavailability was approximately 30%. These disposition and kinetic bifenthrin data may decrease uncertainties in the risk assessment for this pyrethroid insecticide.

Keywords
Bifenthrin, disposition, pharmacokinetics, pyrethroids

Introduction

Human exposure to pyrethroid insecticides has increased since 2000 in the United States principally due to the phase-out of organophosphate insecticides in residential and agricultural settings (Power & Sudakin, 2007). Tulve et al. (2006) detected pyrethroids in indoor surfaces of childcare centers and in surrounding soils of these facilities. Other investigators found pyrethroids on floor wipes of residential homes and apartments (Julien et al., 2008; Stout et al., 2009), indoor dust (Harnly et al., 2009; Quiviró-Alcalá et al., 2011) and on fruits and vegetables (USDA, 2013).

Pyrethroid insecticides are synthetic chemicals and their structure is derived from the botanical insecticides, the pyrethrins (Soderlund et al., 2002). Allethrin, initially synthesized in 1949, was the first pyrethroid prepared for commercial use (Casida, 1980). Allethrin and other first-generation pyrethroids are sensitive to sunlight. Chemists synthetically modified the structures of these pyrethroids to generate others which are more photostable and have greater insecticidal activity. Thus, these later generation pyrethroids are more effective in agricultural applications (Casida, 1980). Among this later generation of pyrethroids is bifenthrin (Figure 1).

Pyrethroids are basically classified into one of two types (Soderlund et al., 2002). Type-I pyrethroids are distinguished from type-II pyrethroids based on the absence or presence, respectively, of an α-cyano group in the alcohol moiety and acute neurobehavioral activity of rats following oral or parenteral administration (Soderlund et al., 2002). Rats exposed to type-I pyrethroids, such as bifenthrin (Figure 1), display aggressive sparring, altered sensitivity to external stimuli and fine tremor progressing to whole-body tremor and prostration. Profuse salivation and writhing seizures are...
displayed in rats exposed to type-II pyrethroids. An example of a type-II pyrethroid is deltamethrin.

It is important to understand the disposition and kinetics of pyrethroid insecticides to assess the potential risks of exposure to these compounds. Pyrethroids studied in rats include deltamethrin (Anadón et al., 1996; Godin et al., 2010; Gray & Rickard, 1982a; Kim et al., 2008; Mirfazaelian et al., 2006; Ruzo et al., 1978), permethrin (Anadón et al., 1991; Gaughan et al., 1977; Tornero-Velez et al., 2012), \( \lambda \)-cyhalothrin (Anadón et al., 2006) and more recently bifenthrin (Gammon et al., 2014). Oral bioavailability of pyrethroids ranges from 25% for deltamethrin (Godin et al., 2010) to 71% for \( \lambda \)-cyhalothrin (Anadón et al., 2006). Once systemically absorbed, pyrethroids are distributed rapidly and widely within the body, being detected in the brain, liver and adipose tissue (Kaneko, 2011; Soderlund et al., 2002). Pyrethroids are cleared from brain and liver but are retained in adipose tissue with half-lives nearing 2–3 weeks. Pyrethroids are metabolized by oxidation and hydrolysis, conjugated and excreted in urine and feces. Absorption, bioavailability and ultimately the neurotoxic effect of pyrethroids are influenced by the vehicle used (e.g. corn oil, glycerol formal) (Crofton et al., 1995; Kim et al., 2007) and vehicle volume (e.g. 1 vs. 5 mL) (Wolansky et al., 2007).

The objective of this study was to evaluate the oral pharmacokinetics including bioavailability of the pyrethroid insecticide bifenthrin in the Long-Evans rat. Our laboratory and collaborators used this rat strain in several neurotoxicology and disposition studies (Crofton et al., 1995; Godin et al., 2010; Scollon et al., 2011; Tornero-Velez et al., 2012; Wolansky et al., 2006, 2007). We administered bifenthrin by oral gavage at two dose levels and studied the tissue disposition of parent compound out to 21 days. Parent compound was measured because laboratory studies suggest it is the neurotoxic entity. Intracerebral administration of pyrethroids in mice and rats results in the classic neurobehavioral endpoints (Gray & Rickard, 1982b; Lawrence & Casida, 1982). The concentration of deltamethrin (Anand et al., 2006; Kim et al., 2010; Rickard & Brodie, 1985) and bifenthrin (Scollon et al., 2011) in brain correlate with neurobehavioral effects in rats. Also, tremors are not observed in rats administered pyrethroid metabolites (White et al., 1976).

**Material and methods**

**Chemicals**

All chemicals used in this study were of analytical grade or better unless otherwise specified. Bifenthrin (CAS no. 82657-0403, 2-methyl-1,1-biphenyl-3-yl-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate, 98% purity) used in dosing solutions and as an analytical standard was from Chem Service (West Chester, PA). The cis isomer of bifenthrin was used in the study, and it is the predominant stereoisomer found in the technical product. Other analytical standards that were used included cis- (99% purity) and trans-permethrin (3-phenoxybenzyl(1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 94% purity) and were also from Chem Service. Labeled cis- and trans-permethrin (phenoxy-\(^{13}\)C\(_6\)) were purchased from Cambridge Isotope Laboratories (Andover, MA) and used as internal and surrogate standards, respectively. Solvents including acetone, hexanes (Fisher Scientific, Pittsburgh, PA) and methanol (VWR, West Chester, PA) were pesticide grade quality. Corn oil, glycerol formal and dextrose were from Sigma (St. Louis, MO), heparin from Baxter Healthcare Corp. (Deerfield, IL) and saline from Abbott Laboratories (North Chicago, IL).

**Animals**

Male Long-Evans rats (Charles River Laboratories, Inc., Wilmington, MA) were obtained at 55–58 days of age and housed two per cage in standard polycarbonate hanging cages (45 cm × 24 cm × 20 cm) containing heat-sterilized pine
shavings (Northeastern Products, Inc., Warrensburg, NY). Jugular-vein cannulated rats (Charles River) were housed singly in the standard cages with pine shavings. Animals were given a 5-9-day acclimation period and were maintained on a 12:12-h photoperiod (L/D, 0600:1800). Feed (Purina 5001 Lab Chow, Barnes Supply Co., Durham, NC) and tap water were provided ad libitum. Tap water (Durham, NC) used in the animal facility was filtered through sand and activated charcoal and rechlorinated to 4–5 ppm Cl\(^{-}\). Animal-holding rooms were maintained at 22 ± 2°C and relative humidity at 50 ± 10% in an Association for Assessment and Accreditation of Laboratory Animal Care approved facility. The Institutional Animal Care and Use Committee of the US EPA’s National Health and Environmental Effects Research Laboratory approved all experimental protocols in advance.

**Experimental**

There were two experiments in this study, a tissue time course (with serial sacrifice) and a serial blood sampling for bioavailability assessment following administration of bifenthrin. For both experiments, rats were administered bifenthrin at a dose of 0.3 or 3 mg/kg in corn oil (1 mL/kg) by oral (po) gavage. Our group has used corn oil as a vehicle for the oral administration of pyrethroids in several studies (Godin et al., 2010; Tornero-Velez et al., 2012; Wolansky et al., 2006, 2007). The high dose of bifenthrin is equivalent to an oral dose that decreases motor activity in a rat by 30% (Wolansky et al., 2006). As part of the bioavailability study, rats were also administered bifenthrin (0.3 mg/kg, 0.1 mL/kg) intravenously in glycerol formal via the jugular vein catheter. After intravenous dosing, the catheter was flushed with 300 μL saline and filled with a void volume of heparinized dextrose to maintain catheter patency. The weight of the rats on the day of dosing (po and iv) ranged from 306.2 to 400.6 gm.

For the tissue time-course study, the animals were sacrificed by cardiac puncture under CO\(_2\)-induced anesthesia at 0.25-, 0.5-, 1-, 2-, 4-, 6-, 8-, 12-, 24-, 48- (2 days), 168- (7 days), 336- (14 days) or 504 (21 days)-h postadministration. Tissues (whole brain, liver and abdominal fat) were collected, weighed and flash frozen in liquid nitrogen and stored at −80°C. For the bioavailability study, serial blood samples were removed (300 μL) via the catheter and immediately flash frozen in liquid nitrogen and stored at −80°C. For the bioavailability study, serial blood samples were removed (300 μL) via the catheter and immediately flash frozen in liquid nitrogen. For the po-dosed animals, blood samples were taken at 0.25-, 0.5-, 1-, 2-, 3-, 4-, 6-, 8-, 12-, 24-, 48- (2 days), 168- (7 days), 336- (14 days) or 504 (21 days)-h postadministration. Tissues (whole brain, liver and abdominal fat) were collected, weighed and flash frozen in liquid nitrogen and stored at −80°C. For the bioavailability study, serial blood samples were removed (300 μL) via the catheter and immediately flash frozen in liquid nitrogen.

For the tissue extraction, bifenthrin followed the procedure of Godin et al. (2010). Frozen whole brain, liver and adipose tissue were homogenized in a Spex CertiPrep 6850 freezer/mill (Metuchen, NJ) apparatus to form a fine homogenous powder. Duplicate samples of pulverized tissue (300–500 mg) and thawed blood (2 mL for time-course study) from the serial sacrifice study were vortex extracted with 20:80 ace tone/hexane. The whole sample of blood (0.3 mL) from the bioavailability study was similarly extracted. About 25 μL of 6 μM labeled trans-permethrin (phenoxy-\(^{13}C\_6\)) in ace tone was added prior to the extraction of all samples as a surrogate. Samples were vortexed for 10 min in 16 × 100-mm glass tubes with 5 mL solvent and centrifuged. The organic fraction was collected. The extraction was repeated two more times with 3 mL solvent. The pyrethroid-containing organic extracts were combined, dried under a stream of nitrogen and reconstituted in 1 mL hexane.

Reconstituted blood and brain extracts underwent solid-phase extraction (SPE) using Sep-pak 500 mg silica SPE columns (Waters, Inc., Milford, MA). The solid-phase cleanup was automated using a RapidTrace SPE Workstation (Hopkinton, MA). SPE columns were washed with 5 mL hexane before sample loading. Analytes were eluted with 5 mL 94:6 hexane/ethyl acetate. Column eluants were dried under a stream of nitrogen and reconstituted in 1 mL of 90:10 methanol/water with 25 μL of 6 μM labeled cis-permethrin (phenoxy-\(^{13}C\_6\)) added as an internal standard of instrument efficiency for HPLC tandem mass spectrometry analysis.

Reconstituted liver and adipose tissue extracts were cleaned using styrene divinylbenzene gel permeation columns (O1 Analytical, College Station, TX) with a 70% ethyl acetate and 30% cyclopentane mobile phase flowing at a rate of 5 mL/min. A 25 mL fraction containing the pyrethroids was collected with a fraction collector and then dried under a stream of nitrogen. Samples were reconstituted in 3 mL hexane. These extracts were partitioned 3x against equal volumes of hexane-saturated acetonitrile. The acetonitrile fractions were combined, dried under a stream of nitrogen and reconstituted in mobile phase with internal standard (25 μL of 6 μM labeled cis-permethrin (phenoxy-\(^{13}C\_6\))).

**Analytical**

Sample analysis was performed using an AB SCIEX (Framingham, MA) model API 4000\(^{3}M\) liquid chromatography-tandem mass spectrometer (LC/MS/MS) system configured with a Turbo Ion Spray. Conditions and settings were the same as those described by Scollon et al. (2011). The mobile phase consisted of methanol/5 mM ammonium acetate in water (98:2) and flowed at a rate of 400 μL/min. An analytical column (C18, 3.5 μm, 150 × 3 mm) from Agilent Technologies (Santa Clara, CA) was used. Two replicates from the tissue time-course study were processed and analyzed for each sample and the results were averaged.

When recovery of the surrogate (i.e. trans-permethrin (phenoxy-\(^{13}C\_6\))) was within 80–120% of the expected value, the value of the sample was considered acceptable. Samples with surrogate recoveries above or below the acceptable range were reanalyzed. If samples were still outside the acceptable range, additional tissue was extracted and analyzed.

For each type of tissue, bifenthrin and the surrogate trans-permethrin (phenoxy-\(^{13}C\_6\)) were quantified using matrix matched six-point calibration curve with cis-permethrin.
(phenoxyl-$^{13}$C$_6$) as the internal standard for both analytes. Matrices for the calibration standards were made by collecting and processing appropriate tissue masses, or volumes, from untreated rats. To provide more precise estimates of tissue concentrations, ranges of bifenthrin concentrations used for a calibration curve were generally limited to two orders of magnitude. All samples were run initially against a curve with bifenthrin concentrations ranging from 1 to 100 ng/mL. Samples with concentrations outside these values were reprocessed using relevant calibration curve concentrations and the minimum and maximum bifenthrin concentrations (all tissues) were 0.1 and 3000 ng/mL. After adjusting for the mass of each tissue that was collected during tissue harvesting (2 mL blood, 0.33 g brain, 0.11 g liver and 0.11 g adipose tissue), the limits of quantitation for bifenthrin were; 0.05 ng/mL blood, 0.3 ng/g brain, 0.88 ng/g liver and 0.93 ng/g adipose tissue.

Data analysis

The data in the graphs are displayed as mean ± SD ($N = 3–4$ rats). Concentration values > 0 but < LOQ were assigned a value of $1/2$ LOQ. Maximal concentration ($C_{\text{max}}$) and the time ($T_{\text{max}}$) occurred were determined by observation of the sampled time points (Supplemental Figures 1–4). Data from the tissue time course study were dose-normalized. Data from each dose and time point were averaged. These data were analyzed by a two-way analysis of variance (ANOVA) with time, dose and time $\times$ dose as factors (GraphPad Prism, version 6.0, San Diego, CA). The level of significance was $p < 0.05$. For a significant dose effect, differences in $C_{\text{max}}$ were analyzed by a Tukey’s multiple comparison test. The level of significance was $p < 0.05$. The kinetic parameters of this normalized data were determined using a noncompartmental method of analysis (PK Solutions, version 2.0, Summit Research Services, Montrose, CO). The following equation was used for this analysis:

$$\text{Concentration} = Ae^{-\alpha t} + De^{-\delta t} + Ee^{-\gamma t}$$

Where A, D, E represent the $y$-axis intercepts and $\alpha$, $\delta$ and $\gamma$ represent the respective rate constants for the phases of absorption, distribution and elimination, respectively.

The analysis for blood, brain and liver were truncated to 24, 48 and 12 h, respectively, in order to make dose comparisons. Area under the curve (AUC) was determined by the trapezoidal rule. Exponential terms were determined by the method of residuals (i.e. curve stripping). The slope of the apparent linear terminal phase was estimated using at least three data points. The elimination rate constant ($k_{\text{el}}$ or $\gamma$) was determined by the following equation:

$$k_{\text{el}} = -2.303 \times \text{slope of the apparent linear terminal phase}$$

The elimination half-life ($t_{1/2}$) was determined by the following equation:

$$t_{1/2} = 0.693/k_{\text{el}}.$$ 

For the analysis of the data from serial blood sampling (i.e. bioavailability study), the kinetic parameters for each animal were determined and then averaged. The parameters were determined using noncompartmental method of analysis (PK Solutions, version 2.0, Summit Research Services, Montrose, CO). The three-term equation described previously was used for the analysis of the oral data and the following equation was used for the intravenous data:

$$\text{Concentration} = Ae^{-\alpha t} + Be^{-\beta t},$$

where A and B equal the $y$-intercepts and $\alpha$ and $\beta$ represent
the rate constants of the distribution and elimination phases, respectively. AUC and area under the moment curve (AUMC) were determined by the trapezoidal rule. Exponential terms were determined by the method of residuals (i.e., curve stripping). The volume of distribution at steady-state ($V_{ss}$) was determined by the following equation:

$$V_{ss} = \text{Dose} \times (\text{AUMC}) / (\text{AUC})^2.$$  

Bioavailability was determined by the following equation:

$$\text{Bioavailability} = \left(\frac{\text{AUC}_{po}}{\text{AUC}_{iv}}\right) \times \left(\frac{\text{Dose}_{iv}}{\text{Dose}_{po}}\right) \times 100.$$  

Clearance (Cl) was determined by the following equation:

$$\text{Cl} = \left(\frac{\text{Bioavailability}}{100}\right) \times \text{Dose} / \text{AUC}.$$  

Statistical analysis of the kinetic parameters of the oral dose bioavailability group was done using a t-test (GraphPad Prism ver.6.0, GraphPad Software, Inc., San Diego, CA) with a level of significance of $p < 0.05$.

**Results**

**Tissue time course following oral administration and serial sacrifice**

The absorption of bifenthrin into the blood following oral administration was rapid (detected within 15 min), peaked early (1 h) and then started to decrease over time (Supplemental Figure 1). Bifenthrin was not detected in blood after 48- and 336-h postadministration for the 0.3 and 3 mg/kg doses, respectively. There was an 11-fold difference in the maximal concentration ($C_{max}$) (Table 1). The dose-normalized concentration–time course of bifenthrin in blood is shown in Figure 2. The two-way ANOVA indicated that there were significant effects on time ($p < 0.0001$). The concentration of bifenthrin in blood for the two doses appears to be within the linear dose range out to 24-h postadministration. The elimination rate constants and half-lives were similar for both normalized doses of bifenthrin (Table 1) and showed the elimination of bifenthrin from blood was rapid. The dose-normalized AUC$_{0-24h}$ of bifenthrin in blood was 9% greater for the low than the high dose (Table 1).

Bifenthrin distributed rapidly to the liver as it was detected within 15-min postadministration by the oral route (Figure 3). The concentration of bifenthrin peaked 1–2-h postadministration for both doses (Table 1). There was an approximate 14-fold difference in bifenthrin $C_{max}$ between the two doses. Bifenthrin in liver was not detected after 12- and 48-h postadministration for the low and high dose, respectively (Supplemental Figure 3). The dose-normalized concentration–time course of bifenthrin in liver is shown in Figure 3. The two-way ANOVA showed that there were significant effects of time ($p < 0.0001$) and the interaction of dose $\times$ time ($p = 0.044$). The concentration of bifenthrin in liver for the two normalized doses appears to be within the linear dose range out to 12-h postadministration. The elimination rate constant for the normalized low dose of bifenthrin was about 50% greater than the normalized high dose (Table 1). The elimination half-life for bifenthrin was 1–2 h, indicating elimination of bifenthrin from the liver was rapid. The dose-normalized AUC$_{0-12h}$ for the high dose of bifenthrin was about 30% greater than that for the low dose (Table 1).

The maximal concentration of bifenthrin in brain after oral administration was lower and did not peak as early as in blood and liver for both doses (Table 1, Supplemental Figure 3). There was a 12-fold difference in bifenthrin $C_{max}$ in the brain between the two doses. The dose-normalized concentration–time course of bifenthrin in brain is shown in Figure 4. The two-way ANOVA showed that there were significant effects on time ($p < 0.0001$), dose ($p = 0.0028$) and the interaction of dose $\times$ time ($p = 0.028$). However, there was no significant difference in the $C_{max}$ between the two doses. The elimination rate constants of bifenthrin in brain were similar, and the elimination half-life was approximately 12–13 h (Table 1). The dose-normalized AUC$_{0-48h}$ for the high dose of bifenthrin in brain was about 35% greater than that for the low dose (Table 1).

Bifenthrin distributed more slowly to the adipose tissue after oral administration and was retained unlike the other tissues (Supplemental Figures 4A and 4B, Table 1). Peak concentrations of bifenthrin in adipose tissue were at the sampled time points of 8 and 24 h for the low and high dose, respectively, and were the highest for each dose level of the tissues analyzed. The dose-normalized concentration–time course of bifenthrin in adipose tissue is shown in Figure 5. The two-way ANOVA indicated that there were significant time effects ($p < 0.0001$). The concentration of bifenthrin in adipose tissue for the two doses appears to be within the linear range out to 506-h postadministration. The elimination rate constants for both normalized doses of bifenthrin were low at 0.001/h (Table 1). The half-life for both doses was high at 693 h, indicating elimination of bifenthrin from adipose tissue was slow. The dose-normalized AUC$_{0-506h}$ for the high dose of bifenthrin was about 9% greater than that for the normalized low dose (Table 1).

**Serial blood sampling following oral and intravenous administration**

Bifenthrin was not detected in the blood collected before dosing the rats with this insecticide. The bifenthrin blood concentration–time course (Figure 6) following iv
The inset shows the concentration–time course to 12 h. Animals were serially sacrificed over time. Data points represent mean ± SD, N = 3–4 (animals).

Following oral administration of bifenthrin and serial blood sampling, peak concentration (Figure 7) was 2 h for the 0.3 mg/kg dose and 1 h for the 3 mg/kg dose. The elimination half-life was about 3 times longer for the low dose than the high dose (Table 2). The clearance values were essentially the same, approximately 100 mL/h, but the volume of distribution at steady state was significantly greater for the low dose (3660 mL) than the high dose (1000 mL) of bifenthrin.

The AUC for bifenthrin in blood in the oral bioavailability study was approximately 266 and 4081 ng-h/mL for the 0.3 and 3 mg/kg doses, respectively (Table 2). There was an approximate 15-fold difference (p < 0.05) in the AUC between these two doses. The iv AUC for bifenthrin in blood was about 1078 ng-h/mL. The bioavailability for the low and high dose of bifenthrin was 25 and 38%, respectively.

Discussion

The mammalian disposition of several type-I and type-II pyrethroids such as permethrin (Anadón et al., 1991; Tornero-Velez et al., 2012) and deltamethrin (Anadón et al., 1996; Godin et al., 2010; Kim et al., 2008), respectively, have been studied. However, information regarding the disposition of many other pyrethroids that are also in use today, such as bifenthrin, are not well characterized. Gammon et al. (2014) recently compared the pharmacokinetics of bifenthrin in the male Sprague-Dawley rat following inhalation, iv and po administration. They analyzed plasma instead of blood for bifenthrin as we report, and after oral administration, they observed lower maximal levels in plasma (361 ng/ml) than with blood (947 ng/ml), but nearly the same levels in brain (83 ng/g) as we report (143 ng/g) in the present study. Data, such as dose to target tissue, which are obtained from chemical disposition and kinetic studies, are needed to allow risk assessors to make informed decisions when evaluating the potential health risk from exposure to pyrethroid insecticides.

Bifenthrin kinetics in the rat have many similarities with pyrethroids such as deltamethrin (Anadón et al., 1996; Godin et al., 2010; Kim et al., 2008), permethrin (Anadón et al., 1991; Tornero-Velez et al., 2012) and \( \pm \)-cyhalothrin (Anadón et al., 2006) after oral administration. The similarities are prompt absorption into the systemic circulation and clearance from it. Pyrethroids, including bifenthrin, are rapidly distributed to tissues such as the liver and brain after oral administration. Bifenthrin was cleared fairly quickly from liver (elimination half-life <3 h). Tornero-Velez et al. (2012) reported lower elimination half-lives for \( cis\)- and \( trans\)-permethrin in liver (<1.5 h) of the rat after oral administration of a 40:60 mixture of \( cis\)-\( trans\)-permethrin (doses of 1 and 10 mg/kg). In brain, the elimination of bifenthrin was slower than observed in the liver; the levels of bifenthrin in brain were detected out to 14 days at the 3 mg/kg dose. Our tissue analysis of bifenthrin did not differentiate between compound in tissue and that in residual blood in tissue. The animals were euthanized by exsanguination under anesthesia, which would result in some blood being removed from the tissues. The area under the curve data (Table 1) indicates the concentration of bifenthrin was 36 to 100% higher in liver than in blood, suggesting that bifenthrin had been absorbed into these tissues. At the 3 mg/kg dose, the concentration of bifenthrin in brain exceeded the concentration in blood at 24-, 48- and 168-h postadministration (Supplemental Figure 5) suggesting that bifenthrin was absorbed into brain. Gammon et al. (2014) reported brain concentrations of bifenthrin after a po dose (3.1 mg/kg) out to 12-h postadministration, whereas we went out to 48 h for the low dose and 336 h (14 days) for the high dose. The brain concentration of bifenthrin at both doses was constant from 4 to 24 h in the present study. In contrast, the elimination of \( cis\)- and \( trans\)-permethrin from brain (elimination half-life ≤5 h) was faster than bifenthrin (Tornero et al., 2012), although levels of \( cis\)-permethrin were detected out to 48 h in this organ. Kim et al. (2008) also detected deltamethrin (10 mg/kg dose) in brain out to 48 h. The levels of bifenthrin in brain began to decrease 24-h postadministration. Peak motor activity effects of bifenthrin.
in the rat occur at 4-h postadministration when the dosing volume is 1 mL/kg (Wolansky et al., 2006, 2007). This is the same volume used in this study. Although we did not measure motor activity in this study, we did not observe any overt signs of toxicity with the doses administered. Gammon et al. (2014) also reported no signs of toxicity in Sprague-Dawley rats after a similar oral dose (3.1 mg/kg) or an equivalent inhalation dose. Although bifenthrin was detected in brain out to 14 days postadministration in the present study, Wolansky et al. (2006) reported that the neurotoxic effects of orally administered pyrethroids in the rat subside 24–48 h postadministration. This suggests that there is a threshold dose of the pyrethroid that must be attained in the brain for an effect to occur. Distribution of bifenthrin and other pyrethroids to adipose tissue takes somewhat longer (up to 24 h) to attain peak concentrations (Godin et al., 2010; Kim et al., 2008; Tornero-Velez et al., 2012); pyrethroids, including bifenthrin, are retained in this tissue. Bifenthrin was detected in adipose tissue out to 21 days postadministration in the present study. Following iv administration, bifenthrin in blood shows a rapid distribution phase followed by a slower elimination phase, and these results are similar as reported by Gammon et al. (2014). Similar kinetic characteristics following iv administration are also observed in the rat with permethrin, deltamethrin and Δ-cyhalothrin (Anadón et al., 1991, 1996, 2006).

Bifenthrin was persistent in adipose tissue out to at least 21 days, which was the final time point in our study. Pyrethroids such as deltamethrin, cis-permethrin and cis-cypermethrin following oral administration persist in adipose tissue of rats and mice (Crawford et al., 1981; Godin et al., 2010; Hutson et al., 1981; Kim et al., 2008; Marei et al., 1982; Tornero-Velez et al., 2012). Pyrethroids have fairly high log octanol/water (Log P) partition coefficients (Laskowski, 2002). Bifenthrin has a measured and calculated Log P of 6.4 and 7.2, respectively (Laskowski, 2002). With its physicochemical preference toward organic phases, it is reasonable that bifenthrin distributes to adipose tissue and accumulates to levels higher than observed in other organs or tissues. Pyrethroids that persist within adipose tissue most likely would not be metabolized, as lipases have no enzymatic activity toward these insecticides (Ross et al., 2006). The primary means of decreasing pyrethroid levels in adipose tissue would be redistribution into the systemic circulation. Once in the systemic circulation of the rat, pyrethroids could be hydrolyzed by serum esterases or taken up by the liver and metabolized by oxidation, hydrolysis or by both processes.

The persistence of pyrethroids in adipose tissue appears to be dependent on the rate of metabolism of the pyrethroid. Pyrethroids with the structure of a primary alcohol ester in the trans configuration tend to be more readily metabolized, particularly by hydrolysis, than their counterpart pyrethroids in the cis configuration (Soderlund & Casida, 1977). Analysis of rat adipose tissue following the administration of cis/trans-permethrin or cis/trans-cypermethrin shows that the cis isomer of these pyrethroids persists to a greater extent in this tissue than the trans isomer (Crawford et al., 1981; Rhodes et al., 1984; Tornero-Velez et al., 2012). In this study, the cis isomer of bifenthrin was used. Bifenthrin in the cis configuration is not hydrolyzed in rat hepatic microsomes (Scollon et al., 2009) and the hydrolytic activity of recombinant carboxylesterases is low toward this pyrethroid compared to trans-permethrin and trans-cypermethrin (Stok et al., 2004; Nishi et al., 2006). The lower rate of metabolism of pyrethroids in the cis configuration suggests that they are more likely to persist in the body, particularly in lipid rich tissues. The differences in metabolism and persistence of the cis/trans pyrethroid isomers could be important if exposures to pyrethroids are being reconstructed by analyzing adipose tissue. Exposure to pyrethroids in the trans configuration would be underestimated, and thus, the actual exposure to the pyrethroid would be underestimated. This would lead to misinformed health risk estimates from exposures to pyrethroid insecticides.

In previous work from our laboratory with deltamethrin (Godin et al., 2010), we observed an approximate twofold lower peak concentration of this pyrethroid in brain as in the present study with bifenthrin, using the same route of administration (oral) and dose (3 mg/kg; molar dose: bifenthrin, 7.1 μmole/kg; deltamethrin, 5.9 μmole/kg). The neurotoxic
effect of the pyrethroids appears to be related to their concentration in brain (Anand et al., 2006; Kim et al., 2010; Rickard & Brodie, 1985; Scollon et al., 2011). Wolansky et al. (2006) reported that bifenthrin had a higher effective dose$_{50}$ than deltamethrin for a comparable decrease in motor activity in the rat. Our bifenthrin brain concentration results support the hypothesis that brain concentrations of pyrethroids are related to neurotoxic effect. The differences in neurotoxic potency of bifenthrin and deltamethrin may be due to differences in pharmacokinetics, pharmacodynamics or both. In both studies, the cis isomer of bifenthrin and deltamethrin was used. This isomer is the predominant isomer found in insecticidal formulations for these two pyrethroids. The higher concentration of bifenthrin in the brain suggests that deltamethrin is more rapidly metabolized. Ghiasuddin and Soderlund (1984) reported on the hydrolysis activity in the soluble fraction of mouse brain homogenates. Greater activity was found in the soluble fraction than the nuclear and microsomal fractions of the brain. In their assays, Ghiasuddin and Soderlund examined seven pyrethroids including cis- and trans-permethrin and deltamethrin. Of these three pyrethroids, the hydrolysis rate of trans-permethrin was about 4- and 8-fold greater than cis-permethrin and deltamethrin, respectively. The liver is an important metabolizing organ of pyrethroids. The hydrolysis activity toward trans-permethrin in mouse liver is 57-fold greater than in brain (Ghiasuddin & Soderlund, 1984). The role of cytochrome P450s in metabolizing pyrethroids in brain is not well characterized. An alternative explanation is that deltamethrin is transported out of the brain more rapidly than bifenthrin, but whether pyrethroids are substrates for transporters in the brain is not known.

It does appear, however, that pyrethroids bind to transporter proteins located in areas outside of the brain. Permethrin and resmethrin, but not cypermethrin and deltamethrin, bind to and inhibit ATPase activity of the human breast cancer resistance protein efflux transporters (Bircsak et al., 2013). None of these same pyrethroids inhibit ATPase activity of the human multidrug resistance protein 1 (mdr1) efflux transporters (Bircsak et al., 2013). The transport of tetrathiomethylrosamine by mdr1 isolated from plasma membrane of a Chinese hamster ovary cell line that is multidrug resistant was inhibited by cypermethrin and fenvalerate (Sreeramulu et al., 2007). Transport of doxorubicin by human mdr1 was weakly inhibited by fluvilinate and permethrin but not fenvalerate (Bain & LeBlanc, 1996). Intestinal transport studies by Zastre et al. (2013) using Caco-2 cells indicate that there is no efflux of deltamethrin or cis/trans-permethrin by mdr1 in experiments with Caco-2 cells. Additional experiments by this group suggest a role for an unspecified influx transporter. However, the permeability coefficients of the pyrethroids tested by Zastre et al. (2013) in their in vitro model system were similar to that of mannitol, which is used as a marker for low permeability and low absorption. Zastre et al. (2013) interpret their results to indicate that deltamethrin and cis/trans-permethrin have low potential for gastrointestinal absorption. Whether this occurs with bifenthrin is not known and should be investigated.

Conclusions

Bifenthrin was rapidly absorbed by the rat following oral administration. It was detected in blood, liver, brain and adipose tissue. Bifenthrin was cleared rapidly from blood and liver, persisted out to about 14 days in brain and was retained in adipose tissue out to 21 days. Bioavailability of bifenthrin was 25–38%. These data will aid risk assessors when evaluating the potential for adverse health effects to humans following exposure to bifenthrin by decreasing uncertainties in the disposition of this pyrethroid pesticide.

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Declaration of interest

The authors declare that there is no conflict of interest. This article is reviewed in accordance with the policy of the National Health and Environmental Effects Research laboratory, U.S. Environmental Protection Agency and the National Institute of Environmental Health Sciences and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This article may be the work product of an employee of the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH); however, the
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Supplementary materials available online
Supplemental Figures 1–5