HHS Public Access

Author manuscript

Toxicology. Author manuscript; available in PMC 2015 December 17.

Published in final edited form as:

Toxicology. 2011 November 28; 290(1): 1–6. doi:10.1016/j.tox.2011.08.002.

Correlation of tissue concentrations of the pyrethroid bifenthrin with neurotoxicity in the rat

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Abstract

The potential for human exposure to pyrethroid pesticides has prompted pharmacodynamic and pharmacokinetic research to better characterize risk. This work tested the hypothesis that blood and brain concentrations of the pyrethroid bifenthrin are predictive of neurotoxic effects. Adult male Long Evans rats received a single oral dose of bifenthrin dissolved in corn oil. Using figure-eight mazes, motor activity was measured for 1 h at 4- and 7-h following exposure to bifenthrin (0–16 mg/kg or 0–9 mg/kg, respectively; n = 4–8/group). Whole blood and brains were collected immediately following motor activity assays. Bifenthrin concentrations in blood and brain were quantified using HPLC/MS/MS. Bifenthrin exposure decreased motor activity from 20% to 70% in a dose-dependent manner at both time points. The relationship between motor activity data and administered dose, and blood and brain bifenthrin concentrations were described using a sigmoidal E_{max} model. The relationships between motor activity and administered dose or blood concentrations were different between the 4- and 7-h time points. The relationship between motor activity and brain concentration was not significantly different between the two time points. These data suggest that momentary brain concentration of bifenthrin may be a more precise dose metric

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Conflict of interest

The authors declare there is no conflict of interest in the content of this work.

for predicting behavioral effects because the relationship between brain concentration and locomotor activity is independent of the time of exposure.

Keywords

Bifenthrin; Motor activity; Brain concentrations; Blood concentrations

1. Introduction

Pyrethroid insecticides are synthetic derivatives of pyrethrins, the natural insecticidal compounds found in the chrysanthemum *Chrysanthemum cinerariaefolum* (Bradberry et al., 2005). Pyrethroids are often classified into two groups based upon chemical structure and neurotoxicological effect. Type I pyrethroids, which lack an alpha-cyano moiety, induce in rats a syndrome consisting of aggressive sparring, altered sensitivity to external stimuli, and fine tremor progressing to whole-body tremor and prostration (T-syndrome). Type II pyrethroids, which contain an alpha-cyano moiety, produce in rats a syndrome that includes pawing, burrowing, salivation, and coarse tremors leading to choreoathetosis (CS-syndrome) (Verschoyle and Aldridge, 1980; Lawrence and Casida, 1982). While this classification is useful in characterizing these chemicals, there are a few pyrethroids that elicit neurotoxic signs of both syndromes (Verschoyle and Aldridge, 1980; Soderlund et al., 2002). In addition, this classification is based on high dose effects and may not relate to low dose effects of pyrethroids (Shafer et al., 2005; Soderlund et al., 2002; Wolansky and Harrill, 2008). Regardless of classification, pyrethroids primarily act by disrupting voltage gated ion channels (Soderlund et al., 2002).

The neurotoxicity of pyrethroids appears to be due to parent compound. Induction of neurotoxicity is rapid following intracerebral administration of pyrethroids in laboratory animals (Lawrence and Casida, 1982; Gray and Rickard, 1982a,b). Brain concentrations of the pyrethroid deltamethrin correlate with neurotoxic endpoints (Rickard and Brodie, 1985; Anand et al., 2006; Kim et al., 2010). Rickard and Brodie (1985) also note that direct injection of phenoxybenzoic acid, a hydrolyzed product of deltamethrin, into rat brain was without neurotoxic effect.

Several studies have examined the relationship between blood or brain pyrethroid concentrations and their neurotoxic effects. In rats, the brain concentration of deltamethrin correlates with the induction of the CS-syndrome 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 brain concentrations were approximately 3 nmol/g for deltamethrin, 3.5 nmol/g for cismethrin and 14.5 nmol/g for bioresmethrin. Sheets et al. (1994) reported brain concentrations of deltamethrin were associated with a neurotoxic response (acoustic startle response) and lethality in rats. More recently, Anand et al. (2006) and Kim et al. (2010) correlated levels of deltamethrin in blood and brain with neurotoxic signs measured as salivation and tremors. Overall, these data suggest that for neurotoxicity,

momentary brain concentration may be a useful dose metric for pyrethroids; however, these studies are limited in dose–response information.

In risk assessments, using a dose metric, that quantitatively describes the relationship between exposure, internal dose, and adverse effect can enhance the accuracy in extrapolation across species (Gentry et al., 2002). In this work, we tested the hypothesis that momentary pyrethroid blood and brain concentration correlates with behavioral changes. Bifenthrin, a Type I pyrethroid, was chosen as a prototype pyrethroid. Metabolites of pyrethroids are thought to be non-neurotoxic (Soderlund et al., 2002), therefore, blood and brain concentrations of bifenthrin were quantified and used as dose metrics. Motor activity was chosen as the behavioral endpoint because it is a low dose effect of Type I pyrethroids (Wolansky and Harrill, 2008). Evaluating the relationship between tissue concentration and behavioral effect will aid in determining if brain or blood concentrations are better descriptors of a “biologically effective” dose than is administered dose. With this type of data, more informative physiologically based pharmacokinetic and pharmacodynamic models can be developed and decrease uncertainties in risk assessments of pyrethroids.

2. Materials and methods

2.1. Chemicals

All chemicals were analytical grade unless otherwise specified. Bifenthrin (CAS no. 82657-04-3,2-methyl-1, 1-biphenyl-3-yl-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate, 98% purity), and cis- (99% purity) and trans-permethrin (3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 94% purity), were purchased from ChemService (West Chester, PA, USA) and used as analytical standards. Technical grade bifenthrin (89% purity) (IUPAC: 2-methyl-3-phenyl-phenyl)methyl 3-(2-chloro-3,3,3-trifluoro-prop-1-en-yl)-2,2-dimethyl-cyclopropane-1-carboxylate; 99% + (*Z*)-(1*R*)-cis isomer), used in dosing solutions, was generously provided by FMC Corporation (Philadelphia, PA, USA). This grade of bifenthrin was used in previous rat neurobehavioral studies (Weiner et al., 2009; Wolansky et al., 2006, 2007). Labeled cis- and trans-permethrin (phenoxy-$^{13}C_6$) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and used as internal and surrogate standards, respectively. Solvents, including acetone, hexanes (Fisher Scientific, Pittsburgh, PA) and methanol (VWR, West Chester, PA, USA), were pesticide grade quality.

2.2. Animals

Male Long Evans rats (Charles River Laboratories, Inc., Wilmington, MA, USA) 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, USA). All 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, USA) and tap water were provided *ad libitum*. Tap water (Durham, NC, USA) used in the animal facility was filtered through sand and activated charcoal, and finally rechlorinated to 4–5 ppm Cl^-. Animal holding rooms were maintained at 22.0 ± 2.0 °C and relative humidity at 50 ± 10% in an American Association

for Accreditation of Laboratory Animal Care approved facility. The US EPA's National Health and Environmental Effects Research Laboratory, Institutional Animal Care and Use Committee approved all experimental protocols in advance.

2.3. Experimental procedures

Rats (n = 4 or 8/group) were exposed by gavage to vehicle (corn oil) or bifenthrin at a volume of 1 ml/kg between 08:00 and 10:00 h. At 4 (dose = 0, 0.1, 1, 2, 4, 6, 8, 12, 16 mg/kg) or 7 h (dose = 0, 0.05, 0.5, 1, 3, 4.5, 6, 9 mg/kg) post-dosing, rats were transferred to the testing room in individual plastic cages with pine shavings. The test room was maintained at the same environmental conditions as the animal facility. The use of corn oil as a vehicle and the 4- and 7-h time points were based on previous studies with bifenthrin (Wolansky et al., 2006, 2007). The dose range chosen in this study was designed to optimize the dose response relationships at each time point, based on pilot studies. While some of the doses do not overlap between the two time points, two of the doses were run in both time points.

2.4. Motor activity

Motor activity was measured in 16 figure-eight mazes, as modified by Wolansky et al. (2007). Briefly, motor activity was monitored for 1 h using 16 figure-eight mazes, each consisting of a series of interconnected alleys (10 cm × 10 cm) converging on a central arena and covered with transparent acrylic plastic. After 5 min of acclimation in the testing room, rats were placed into individual mazes. Horizontal and vertical activities were detected by eight phototransistor/photodiode pairs, equally spaced along the maze alleys at 0.5 in. above the floor (horizontal), and four pairs located 3.0 in. above the floor in the central arena. Each time a photobeam was interrupted, an activity count was registered (sampling rate = 1 kHz). Total activity was calculated as the sum of horizontal and vertical activity counts over the 1 h test session. Exhaustive dose–effect studies examining eleven pyrethroids, including bifenthrin (Wolansky et al., 2006, 2007), indicated that there is no relevant advantage by using either component of general activity (i.e., horizontal or vertical movements) as an endpoint to describe pyrethroid actions on figure-eight maze performance. Photobeam calibration was checked daily prior to testing.

2.5. Tissue collection

Blood and whole brain tissue were collected from experimental animals under deep CO_2-induced anesthesia 20–25 min after the motor activity assay was completed. Blood (7–9 ml total) was collected by cardiac puncture, separated into 2 ml aliquots and frozen in a methanol/dry ice bath. Whole brains were removed, flash frozen in liquid nitrogen, and stored at −80°C until homogenized in a Spex CertiPrep 6850 freezer/mill apparatus (Metuchen, NJ, USA). Following homogenization, samples were again flash frozen and stored at −80°C until bifenthrin residue analysis was begun.

2.6. Tissue extraction

Aliquots of thawed blood (2 ml) and brain homogenate (300 mg) were extracted with 20:80 acetone:hexane. Twenty-five μl of 6 μM trans-permethrin (phenoxy-$^{13}C_6$) in acetone was

added prior to extraction as a surrogate of recovery. Samples were vortexed for 10 min in 16 mm × 100 mm culture tubes with 5 ml solvent and then centrifuged at 4000 × *g* for 10 min. The organic layer was collected. The extraction procedure was repeated, and both organic fractions were combined. Bifenthrin-containing organic fractions were dried under nitrogen and reconstituted in 1 ml hexane. Samples were then loaded onto a hexane rinsed Sep-pak 500 mg silica solid phase extraction (SPE) columns (Waters, Inc., Milford, MA, USA). SPE columns were washed with 5 ml hexane and the bifenthrin eluted with 5 ml 94:6 hexane:ethyl acetate. Column eluants were dried under a stream of nitrogen, and reconstituted in 1 ml 90:10 methanol:water with 25 µl of 6 µM cis-permethrin (phenoxy-$^{13}C_6$) in acetone added as an internal standard of instrument efficiency for LC/MS/MS for analysis. The solid phase cleanup was automated using a RapidTrace SPE Workstation (Hopkinton, MA, USA).

Extraction integrity was monitored by adding trans-permethrin (phenoxy-$^{13}C_6$) as a surrogate to each sample prior to extraction (see above). When recovery of the surrogate was within 80 – 120% of the expected value was considered acceptable and required no correction. Samples with surrogate recoveries above or below the acceptable range were reanalyzed.

2.7. Residue determination

Analysis of bifenthrin and $^{13}C_6$-cis- and $^{13}C_6$-trans-permethrin (the internal standard and surrogate, respectively) was performed using an Applied Biosystems API 4000 HPLC/MS/MS (Foster City, CA, USA) operated with the turbo ion spray operated at atmospheric pressure. Positive ionization was used for all compounds and transition ion pairs and retention times were used to verify the identities of analytes. Samples were introduced with an isocratic 98:2 methanol:5 mM ammonium acetate mobile phase flowing at a rate of 0.4 ml/min and separated using a Zorbax 3.5 µm 3.0 mm × 150 mm C18 column (Agilent Technologies, Santa Clara, CA, USA).

Bifenthrin and the surrogate trans-permethrin were quantified using a five-point calibration curve, prepared in sample matrix, containing $^{13}C_6$-cis-permethrin as an internal standard. Residue concentrations were determined by the ratio of internal standard response to the analyte response. Calibration standards ranged from 0.1 to 3000 ng/ml. However, curves were generally limited to two orders of magnitude. Calibration standards were made by adding the appropriate amount of bifenthrin to tissue extracts, cleaned and reconstituted in 1 ml of methanol:water. The concentration of the lowest calibration standard was 0.1 ng/ml that corresponded to tissue concentrations of 0.05 ng/ml and 0.3 ng/g for blood and brain, respectively. The signal to noise ratio of this standard was approximately 20× the standard deviation of noise in unspiked blank matrices. The lowest calibration standard was used as the limit of quantitation. Tissue concentrations less than 0.05 ng/ml (blood) and below 0.33 ng/g (brain) were not quantified.

2.8. Statistical analysis

Linear and non-linear regression analyses were used to assess the relationships between administered dose, blood and brain concentrations of bifenthrin and behavioral effects at the

different time points using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego California, USA, www.graphpad.com). The level of significance was set at $p < 0.05$. Linear regression analysis was used to compare the relationship between administered dose and blood and brain concentrations between the 4- and 7-h groups. Potential differences between the slopes and intercepts of these relationships between the 4- and 7-h groups were evaluated using a *t*-test. A sigmoidal E_{max} model was used to relate motor activity changes to administered dose or tissue concentrations and is given as:

$$y = E_0 \pm \left[\frac{E_{\max} \times X^n}{b^n + X^n} \right].$$

The equation with the negative sign was used in the motor activity analysis. For this analysis, X is the dose, E_0 is the estimated motor activity when X is 0, E_{max} is the estimated maximum possible decrease, b is the estimated ED_{50}, and n is a shape parameter. When n equals 1, the dose–response relationship is linear. When n is less than 1 the dose–response relationship is supralinear. When n is greater than 1 the dose–response relationship becomes threshold-like. The biologically important characteristics of this function are that it has lower and upper extremes that are determined by the biological response independent of dose. In the motor activity modeling exercises, E_0 was constrained at less than 130 and E_{max} was constrained at less than 100. Comparisons between the 4- and 7-h groups used an extra sum of squares *F*-test. The sigmoidal E_{max} model using the positive sign was fit to the bifenthrin blood vs. brain concentration data. In this case, E_0 was set at zero, since in unexposed animals there is no bifenthrin in either blood or brain. Data from rats with either blood or brain bifenthrin concentrations below the level of detection were not included in the analysis.

Bifenthrin blood:brain concentration ratios were compared using a one-way ANOVA with dose as the independent variable for each time point. Comparison of the average blood:brain concentration ratios between the 4- and 7-h groups was performed using a Student's *t*-test.

3. Results

3.1. Exposure–dose relationships

Linear models were used to determine the relationship between administered dose (i.e., exposure) and tissue concentrations (i.e., internal dose) of bifenthrin. A linear model significantly ($p < 0.001$) fit the administered dose vs. blood concentration data for both the 4- and 7-h time points (Table 1; Fig. 1A). The linear model underestimated the blood concentrations in the low dose region at the 4-h time point. While a linear model fit both time points, the slopes were significantly different between the two time points ($F_{1,84} = 19.5$; $p < 0.0003$). The slope of the 4-h group (24.9 ± 3.1; mean ± SE; $n = 50$) was approximately 5 times greater than that of the 7-h group (4.6 ± 0.8; $n = 38$). A linear model also fit the administered dose vs. brain concentrations data for both the 4- and 7-h time points. The slope of the relationship between administered dose and brain concentrations of bifenthrin was not significantly different ($F_{1,85} = 3.7$; $p = 0.058$) between the 4- (32.8 ± 1.5) and 7-h (27.9 ± 1.7) time points and a single equation fits both data sets (Table 1; Fig.

1B).Thus, the relationship between administered dose and brain concentrations of bifenthrin is similar for both the 4-and 7-h time points.

There were no dose-dependent differences in the brain:blood ratios of bifenthrin at 4 ($F_{7,42}$ = 1.8; p = 0.12) and 7 h ($F_{6,37}$ = 1.35; p = 0.27) (Fig. 2A and B).The brain:blood ratio in the 7-h animals was significantly higher than the 4-h group (t-test, p<0.01), 6.0 ± 3.3 vs. 2.6 ± 1.4 (mean ± SD), respectively. The greater ratio in the 7-h group can be attributed to the lower blood concentrations in the rats at this time point (Fig. 1A) and similar brain concentrations at the two time points (Fig. 1B).

The relationship between bifenthrin brain and blood concentrations was evaluated using the sigmoidal E_{max} model. The fits of this model to the 4- and 7-h groups were significantly different ($F_{3,88}$ = 7.6; p < 0.0001) (Table 2, Fig. 1C). The shape parameter of the model at the 4-h time point was close to unity, suggesting a linear relationship between blood and brain concentrations. At the 7-h time point, the shape parameter of the model was estimated at 2.1 ± 1.1, suggesting a slight non-linear relationship between blood and brain concentrations. These data demonstrate that the relationship between brain and blood concentration of bifenthrin is dependent upon the time of exposure.

3.2. Target tissue dose–effect relationships

The fit of the sigmoidal E_{max} model to the administered dose vs. motor activity (MA) data was significantly different ($F_{4,84}$ = 3.8; p = 0.0068) between the 4- and 7-h groups (Table 3, Fig. 3A). The difference between the two time points was in the ED_{50}, with the 4-h time point approximately 30 times lower than the 7-h time point (0.24 vs. 6.0, respectively). In addition, the shape parameter of the dose–response relationship for the 4-h group was approximately 3 times higher than that for the 7-h group, 2.0 vs. 0.6, respectively.

The relationship between bifenthrin blood concentrations and motor activity was also significantly different for the two time points ($F_{4,87}$=4.7; p = 0.0019) (Table 4; Fig. 3B). The value of the shape parameter, n, when near unity suggests a linear relationship, while the shape of the dose–response relationship becomes increasingly threshold-like as n increases. The shape of the 4-h data was more threshold-like compared to the 7-h data based on the estimated shape parameter of 3.3 vs. 0.3, respectively. These data suggest that the relationship between bifenthrin blood concentrations and behavior is different between the two time points.

The sigmoidal E_{max} model fits to the 4- and 7-h bifenthrin brain concentration vs. motor activity data (Table 4; Fig. 3C) were not significantly different from each other ($F_{4,90}$ = 2.0; p = 0.11). Therefore, the two data sets were combined and analyzed. The sigmoidal E_{max} model fit had a shape parameter of 1.47, suggesting slight non–linearity. These results indicate that the relationship between brain concentrations of bifenthrin and behavior is consistent between the two time points.

4. Discussion

This study demonstrates that administered dose and blood and brain concentrations of bifenthrin are predictors of behavioral change in rats. However, the best predictor of these three dose metrics is bifenthrin brain concentration, in part because within the parameters of the experiment (i.e., 4- and 7-h time points), it was independent of time of exposure. In contrast, the relationship between administered dose and blood concentrations of bifenthrin with motor activity are time dependent. The results suggest that momentary brain concentration of bifenthrin is an important dose metric in the prediction of neurotoxicity in rats and may be useful for cross-species extrapolations. Furthermore, these findings are consistent with data from other studies (Anand et al., 2006; Gray et al., 1980; Kim et al., 2010; Rickard and Brodie 1985; Sheets et al., 1994; White et al., 1976) in which concentrations of parent pyrethroids were associated with behavioral effects.

In the first several hours after an oral exposure to a chemical, its concentration in blood can change because of the dynamics of this compartment. Concentrations of chemicals in the blood are sensitive to absorption, distribution and metabolic and renal clearance. Depending on the volatility of the chemical, pulmonary clearance may also be an important factor. Following the absorption and distribution phases, the concentrations of chemicals in blood is less dynamic. In the present study, blood bifenthrin concentration was related to administered dose in a time-dependent manner. The 4-and 7-h groups have different slopes when administered dose was used to predict blood concentration. There was a significant drop in blood levels, between the 4- and 7-h time points, which was likely due to the distribution of bifenthrin into tissues and metabolic clearance of bifenthrin.

Motor activity is a measure of acute bifenthrin toxicity. In this study, bifenthrin exposure produced a dose-dependent decrease in motor activity at both 4 and 7 h after treatment. This is consistent with previous studies that demonstrated a dose-dependent reduction in motor activity in rats following acute oral doses of pyrethroids such as deltamethrin, cypermethrin, permethrin, cismethrin, fenvalerate, and cyfluthrin (Crofton et al., 1995; Crofton and Reiter, 1984, 1987, 1988; Gilbert et al., 1990; McDaniel and Moser, 1993; Wolansky et al., 2006). Bifenthrin was more potent at the 4- than at the 7-h time point and there was no difference in the relationship between administered dose and bifenthrin brain concentrations between the 4- and 7-h time points. The relationship between brain concentration and decreased motor activity was equivalent between the two time points and indicates that brain concentrations are a better predictor than blood concentrations and administered dose of the acute behavioral effects of bifenthrin.

Several studies suggest that toxicity from pyrethroids occur when central nervous system pyrethroid concentrations exceed threshold quantities. Threshold estimates for deltamethrin from iv exposures in rats range from 100 to 500 ng/g brain tissue (Gray and Rickard, 1981, 1982a,b). Tremors and choreoathetosis were observed in rabbits when brain levels of the Type II pyrethroid cypermethrin reached 0.7 ng/g (Khanna et al., 2002). Type I pyrethroids cismethrin and bioresmethrin, administered orally and iv to rats, were much less toxic, requiring 500–1200 ng/g and 5000 ng/g, respectively, for effects to be observed (Gray et al., 1980; Gray and Rickard, 1982a; White et al., 1976). The studies examining tissue

concentrations and behavior focused on high dose behavioral changes such as tremors, choreoathetosis and salivation. According to the present study, brain bifenthrin concentration corresponding to a 50% decrease in motor activity was approximately 289 ng/g (Fig. 3C). The data from the present study are consistent with earlier studies suggesting that higher concentrations of the Type I pyrethroids are necessary to produce behavioral toxicities than the Type II pyrethroids. However, as the sodium channel, the proposed target of the pyrethroids (Soderlund et al., 2002), is present in the central and peripheral nervous system, our results cannot rule out a role for an effect of bifenthrin on the peripheral nervous system.

This study examined the relationships between exposure (oral administration), dose (tissue concentration), and response (motor activity) of bifenthrin. Administered dose and blood and brain concentration can be used as dose metrics to estimate the effects of low dose, environmentally relevant, bifenthrin concentrations on motor function. In the present study the relationship between brain concentration and locomotor activity is independent of time. In contrast, the relationships between administered dose and locomotor activity and bifenthrin blood concentrations are different at the two time points. This suggests that momentary brain concentrations may be a better predictor of the behavioral toxicity of bifenthrin than are blood concentrations and administered dose. Knowing the concentration of toxicant at the target site and confidently relating it to effect can result in risk assessments with lower uncertainties than just using administered dose.

Acknowledgments

Funding

M.J.W. held a National Research Council Research Associateship Award at the National Health and Environmental Research Laboratory, U.S. Environmental Protection Agency while performing this work. Intramural resources of the U.S. Environmental Protection Agency also provided funding.

The authors thank Janet Diliberto and Drs. Linda Birnbaum, Woodrow Setzer, Jane Ellen Simmons and William Mundy for their helpful comments on an earlier version of the version of this manuscript. The authors also thank Drs. N. Hilton and M. Weiner, FMC Corp, Princeton, NJ, for graciously providing the technical grade bifenthrin. The authors also thank Brenda Edwards and Heather Wheeler, for their technical assistance. The data presented in this publication are freely available from the corresponding author (MDV).

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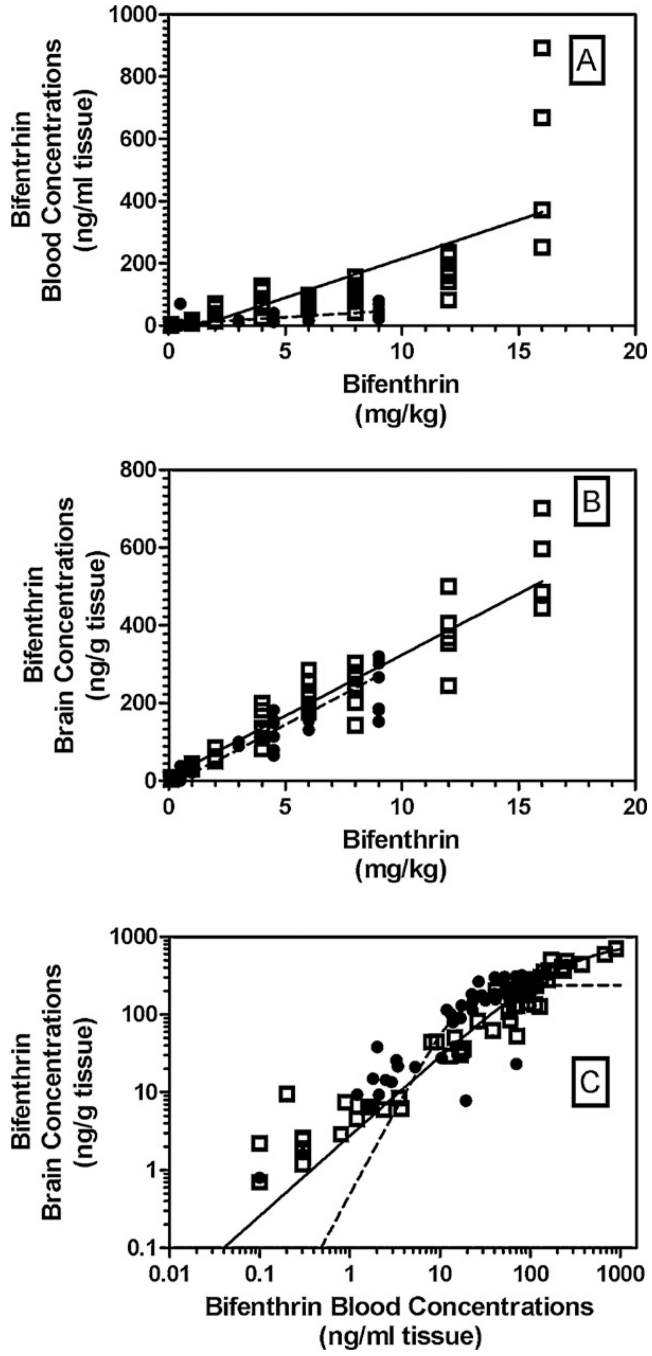

Fig. 1.

The relationship between administered dose of bifenthrin and blood (A) and brain (B) concentrations and blood and brain concentrations (C) of bifenthrin at 4 and 7 h after the oral administration of chemical to male rats. Linear and non-linear models were fit to the 4-h (□, dashed line) and 7-h (●, dotted line) data.

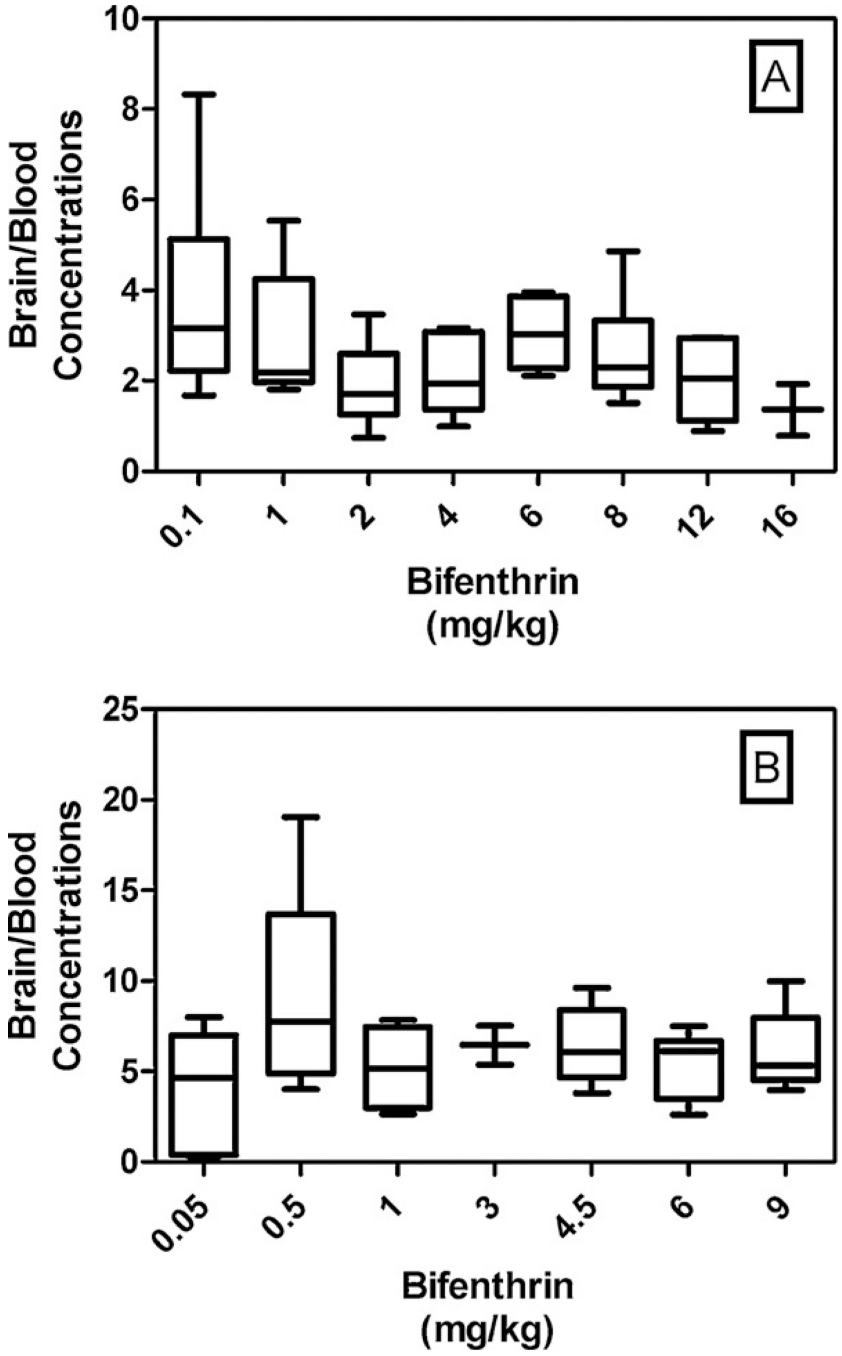

Fig. 2.

The relationship between brain/blood concentration ratios and administered dose at 4 (A) and 7 h (B) in rats treated orally with bifenthrin. The data are presented as a box and whisker format. For each dose level, data are displayed as the lowest, the lower quartile, median, upper quartile and largest ratio observed.

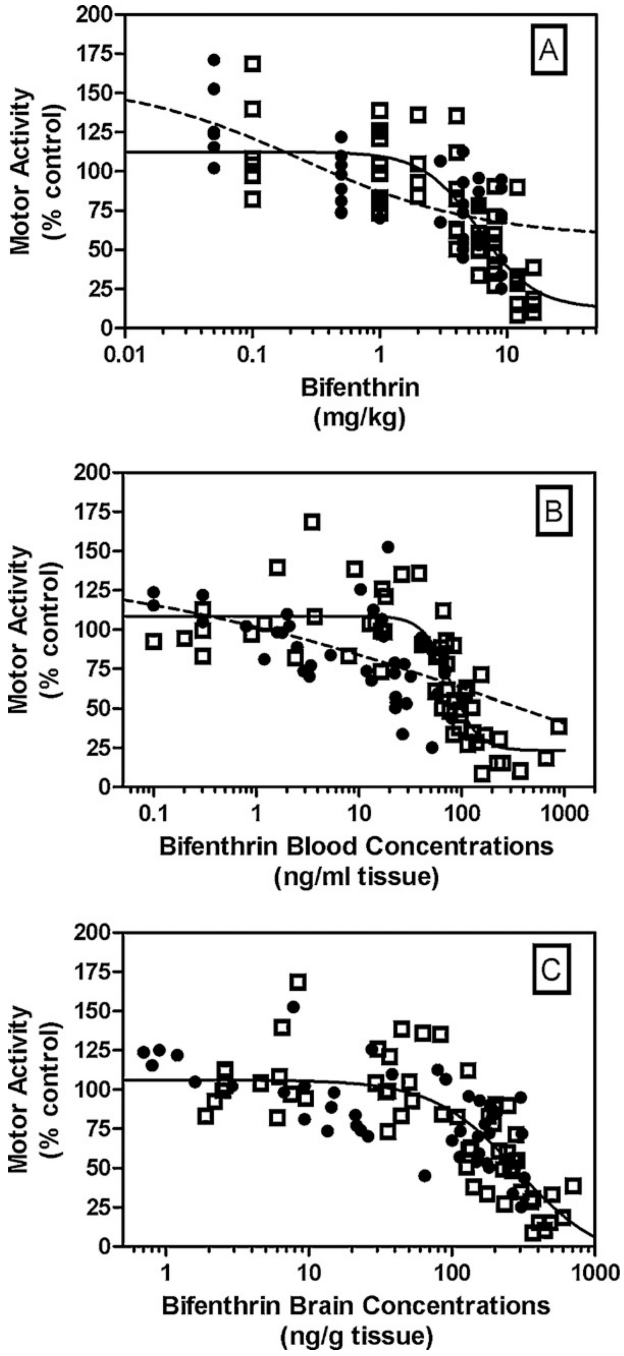

Fig. 3.

The relationship between administered dose (A), blood (B) and brain (C) concentrations and motor activity at 4 and 7 h after the oral administration of bifenthrin to male rats. Non-linear models were fit to the 4-h (□, solid line), 7-h (●, dotted line) and combined data (solid line). In part (C), a single model fit all the data and is represented by a solid line.

Table 1

Linear fits and parameter estimates of bifenthrin tissue concentration vs administered dose at 4- and 7-h post-exposure.

Parameter	Blood concentration vs. administered dose		Brain concentration vs. administered dose	
	4 h	7 h	4 h	7 h
Slope	24.9 ± 3.1[a]	4.6 ± 0.8	32.8 ± 1.5	27.9 ± 1.7
Intercept	−34.5 ± 23.1	4.1 ± 4.2	2.3 ± 11.3	0.2 ± 8.6
r^2	0.55	0.47	0.91	0.88
N[b]	50	38	50	39

[a] Mean ± SE.

[b] Number of animals where tissue concentrations were above detection limit.

Table 2

Non-linear fits and parameter estimates of the bifenthrin blood vs. brain concentrations data at 4- and 7-h post-exposure.[a]

Parameter	Bifenthrin blood vs. brain concentration	
	4 h	7 h
E_{max}	860 ± 147[b]	238 ± 43
n[c]	1.0 ± 0.2	2.1 ± 1.1
b[d]	261 ± 97.5	17.8 ± 4.3
r^2	0.90	0.67
N[e]	58	36

[a] Data were fit with a sigmoidal E_{max} model. E_0 is the concentration of bifenthrin in the brain when the blood concentration was zero. Since there is no chemical in the brain when there is no chemical in the blood, E_0 was fixed at zero for this analysis.

[b] Mean ± SE.

[c] Shape parameter.

[d] Estimated ED_{50}.

[e] Number of animals in which tissue concentrations were above detection limit.

Table 3

Non-linear fits and parameter estimates of the administered dose of bifenthrin vs. motor activity data.[a]

Parameter	4 h	7 h
E_0	112 ± 7.6[b]	158 ± 182
E_{max}	≈100	≈100
n[c]	2.0 ± 1.1	0.6 ± 1.8
b[d]	0.2 ± 1.2	6.0 ± 2.1
r^2	0.66	0.51
N[e]	50	42

[a] Data were fit with a sigmoidal E_{max} model. E_0 is the motor activity of the rats when the administered dose of bifenthrin is zero and E_{max} is the maximal change from control. E_0 was bound at 130 and E_{max} was bound at 100 in order to better fit the model to the data.

[b] Mean ± SE.

[c] Shape parameter.

[d] Estimated ED_{50}.

[e] Number of animals in which tissue concentrations were above detection limit.

Table 4

Non-linear fits and parameter estimates of the bifenthrin blood and brain concentration vs. motor activity data at 4 and 7 h post-exposure.[a]

Parameter	Blood concentrations		Brain concentrations Combined data
	4 h	7 h	
E_0	108.6 ± 4.6[b]	130 ± 129	106.3 ± 4.5
E_{max}	85.2 ± 10.5	≈100	116.5 ± 54.2
n[c]	3.3 ± 1.2	0.34 ± 1.6	1.5 ± 0.6
b[d]	78.5 ± 8.7	15.8 ± 231	289.8 ± 186.7
r^2	0.66	0.12	0.58
N[e]	55	40	98

[a] Data was fit with a sigmoidal E_{max} model. E_0 was the motor activity of the rats when the tissue concentration was zero and E_{max} is the maximum change from controls. E_0 was bound at 130 and E_{max} was bound at 100 in order to better fit the model to the data.

[b] Mean ± SE.

[c] Shape parameter.

[d] Estimated ED_{50}.

[e] Number of animals in which tissue concentrations were above detection limit.