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Environmentally-Relevant Mixtures in Cumulative Assessments: An Acute Study of Toxicokinetics and Effects on Motor Activity in Rats Exposed to a Mixture of Pyrethroids.

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Running Title: **Pyrethroid Kinetics and Effect on Motor Activity.**

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Abstract

Due to extensive use, human exposure to multiple pyrethroid insecticides occurs frequently. Studies of pyrethroid neurotoxicity suggest a common mode of toxicity and that pyrethroids should be considered cumulatively to model risk. The objective of this work was to use a pyrethroid mixture that reflects human exposure to common pyrethroids to develop comparative toxicokinetic profiles in rats, then model the relationship between brain concentration and motor activity. Data from a national survey of child care centers were used to make a mixture reflecting proportions of the most prevalent pyrethroids: permethrin, cypermethrin, β-cyfluthrin, deltamethrin, and esfenvalerate. The mixture was administered orally at one of two concentrations (11.2 and 27.4 $mg{*}kg^{-1}$) to adult male rats. At intervals from 1-24 hours, motor activity was assessed and the animals sacrificed. Pyrethroid concentrations were measured in blood, liver, fat, and brain. After controlling for dose, there were no differences in any tissue concentrations, except blood at the initial time point. Elimination half-lives for all pyrethroids in all tissues were < 7 hours. Brain concentrations of all pyrethroids (when *cis* and *trans*-permethrin were pooled) at the initial time point were proportional to their relative dose. Decreases in motor activity indicated dose additivity and the relationship between pyrethroid brain concentration and motor activity was described by a four parameter sigmoidal E_{max} model. This study links environmental data with toxicokinetic and neurobehavioral assays to support cumulative risk assessments of pyrethroid pesticides. The results support the additive model of pyrethroid effect on motor activity and suggest that variation in the neurotoxicity of individual pyrethroids is related to toxicodynamic rather than toxicokinetic differences.

Keywords: Pyrethroids, Toxicokinetics, Cumulative Risk, Motor Activity.

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Introduction

Pyrethroid pesticides are some of the most commonly applied residential use insecticides in the United States (U.S.), and survey data have repeatedly demonstrated the occurrence and co-occurrence of pyrethroids in residences and child care facilities (Morgan *et al.*, 2004; Stout *et al.*, 2009; Tulve *et al.*, 2006). Their presence in these locations is of concern because children spend the majority of their time indoors (Graham and McCurdy, 2004), may be more susceptible than adults to pyrethroid induced health effects (Tornero-Velez *et al.*, 2010), and non-dietary ingestion of pyrethroids from indoor sources is an important exposure pathway for children (Morgan *et al.*, 2007).

Pyrethroids have 1-3 chiral carbons and are typically divided into two groups, dependent upon the presence or absence of a cyano group at the α-carbon of the alcohol moiety. Both groups are neurotoxicants in mammalian test species but have different high dose acute primary effects. In general, pyrethroids lacking the α-cyano group cause tremors (Type I or T), while α-cyano pyrethroids produce a salivation/choreoathetosis syndrome (Type II or CS). Both types primarily disrupt nervous system function by prolonging the opening of voltage sensitive sodium channels (Narahashi *et al.*, 1998; Soderlund *et al.*, 2002), although kinetic differences between the two types have been noted (Soderlund and Bloomquist, 1989).

Studies of motor activity (Wolansky *et al.*, 2006; Wolansky *et al.*, 2009), functional observational battery (Weiner *et al.*, 2009), and ion channel disruption (Breckenridge *et al.*, 2009), have established that induced symptoms of neurotoxicity vary among the individual

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6 22 pyrethroids. In the motor activity studies, Wolansky *et al.* (2006) ranked the relative potency
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8 23 (RP) of several pyrethroids and demonstrated their dose-additivity (Wolansky *et al.*, 2009).
9
10 24 Citing the importance of the shared effect on sodium channels and the additive effect on motor
11
12 25 activity, the U.S. EPA currently proposes that Type I and II pyrethroids share a common
13
14 26 mechanism of toxicity and therefore present a cumulative risk (U.S. EPA, 2011) under the Food
15
16 27 Quality Protection Act (FQPA, 1996).
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18 28
19
20 29 Because pyrethroids co-occur and can act additively, it is useful to establish toxicokinetic and
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22 30 neurotoxicity data reflecting the collective nature of the exposure. To date, most pyrethroid
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24 31 toxicokinetic reports are from single analyte studies (Anadón *et al.*, 1991; Anadón *et al.*, 1996)
25
26 32 (Anadón *et al.*, 2006; Godin *et al.*, 2010; Hutson and Logan, 1986; Kim *et al.*, 2008; Ohkawa *et*
27
28 33 *al.*, 1979) and comparison of the results is problematic because of differences in doses, vehicles,
29
30 34 and routes of administration (Crofton *et al*., 1995). Further, these studies did not include a
31
32 35 neurotoxicity assessment. Single analyte toxicokinetic assessments by White *et al.* (1976) and
33
34 36 Scollon *et al.* (2011) included neurotoxicity endpoints, but differences in study design and
35
36 37 objectives severely limit comparison of the results. Multi-pyrethroid toxicokinetic profiles such
37
38 38 as that by Marei *et al.* (1982) did not assess neurotoxicity or incorporate exposure related
39
40 39 concepts such as route and relative concentration in the study design. A literature review
41
42 40 revealed no multi-pyrethroid toxicokinetic studies that included an effects endpoint.
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42 The objectives of this research were to 1) use a mixture of pyrethroids that are most frequently
43 detected in an indoor environment to develop comparative toxicokinetic profiles of individual
44 pyrethroids in selected rat tissues, 2) evaluate whether pyrethroid toxicokinetics explain
45 differences in RP reported by Wolansky *et al.* (2006; 2009), and 3) model the relationship
46 between pyrethroid brain concentration and acute motor activity. A pyrethroid mixture was
47 constructed using data from a national probabilistic sampling of care centers (Tulve *et al*., 2006)
48 to determine the identity and relative proportions of the pyrethroids in the mixture. The
49 pyrethroids selected were: permethrin, cyfluthrin, cypermethrin, deltamethrin, and esfenvalerate.
50 Rats were divided into two dose groups and dosed orally at one of two levels with the total
51 pyrethroid concentration in the groups equal to 1.5× (low dose) or 3.7× (high dose) the ED30
52 (Effective Dose30 - dose resulting in a 30% motor activity decrease) assuming dose-addition
53 (Wolansky *et al*., 2009). This research connects the multiple pyrethroids found in child care
54 facilities with dose, toxicokinetics, target organ concentrations, and acute effects. This is a novel
55 approach to the study of chemical mixtures that links exposure science with toxicology.
56

57 **Materials and Methods**

58 **Identification and Formulation of Pyrethroids for Dosing Mixture**

59 The methods used to select the pyrethroids for this study have been described (Tornero-Velez *et*
60 *al*., 2011). Selection was based on a national study (Tulve *et al*., 2006) of a randomly selected
61 set of 168 child care centers from across the U.S. Data for a set of 15 pyrethroids and pyrethrins
62 from indoor surface wipe floor samples were used. For each center, the fractional surface
63 loading (FSL) of each pyrethroid was determined. For each pyrethroid species, its specific FSL

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64 was averaged across the centers. Many samples had non-detectable pyrethroid levels so the
65 analysis was limited to centers with higher pyrethroid surface loadings. To do this, the centers
66 were sorted by total pyrethroid surface load ($ng_{*}cm^{-2}$) and the top 10% of centers (17 centers)
67 identified. In the 17 centers, six pyrethroids accounted for 96.4% of the total pyrethroid surface
68 loaded mass . Normalized by these 6 pyrethroids, the average FSLs were: cypermethrin (0.288),
69 deltamethrin (0.034), esfenvalerate (0.027), *cis*-permethrin (0.198), *trans*-permethrin (0.324),
70 and β−cyfluthrin (0.129).
71
72 Using these values to apportion the pyrethroids, two dose mixture groups were constructed so
73 that the total pyrethroid dose administered to each group was equal to 1.5× (low dose) or 3.7×
74 (high dose) the ED_{30}. These levels were chosen because both doses were expected to result in
75 measurable concentrations of the pyrethroids in all tissues for at least eight hours, and also be
76 disparate enough to result in tissue concentrations that were significantly different (between
77 dose levels) to establish whether the toxicokinetics were dose dependent or independent. In
78 addition, both dose groups were expected to have measurable loss of motor activity but not
79 exhibit the high dose acute primary effects of pyrethroid toxicity. The concentrations of the
80 pyrethroids used in this study and their proportions in the dose mixtures are listed in Table 1.
81 The RP (Wolansky *et al.*, 2006) and the group identity of each pyrethroid (Type I or II) are also
82 listed in Table 1. The relative toxicity of each pyrethroid in the mixtures was calculated by
83 multiplying the percent of total dose for each pyrethroid in the dosing mixtures by its RP. The

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6 84 resulting order expressed as toxicity equivalents was: permethrin < deltamethrin ≈ esfenvalerate
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8 85 < cypermethrin < β-cyfluthrin.
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11 86
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13 87 **Chemicals and Standards**
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15 88 All chemicals used in this study were screened for pyrethroid contamination. Acetone, hexanes,
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17 89 ethyl acetate, methanol (Fisher Scientific, Pittsburgh, PA), cyclopentane and acetonitrile
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19 90 (Honeywell Burdick & Jackson, Muskegon, MI) were pesticide grade or better. All water used
20
21 91 for sample analysis was 18 MΩ resistance. Primary calibration standards including c*is*-
22
23 92 permethrin (99 %), *trans*-permethrin (94 %), deltamethrin (99 %), cypermethrin (98 %),
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25 93 cyfluthrin (98 %) and esfenvalerate (98 %), were purchased from Absolute Standards (Hamden,
26
27 94 CT). Ring-labeled (phenoxy-$^{13}C_6$) pyrethroids used as internal standards or surrogates were
28
29 95 purchased from Cambridge Isotope Laboratories (Andover, MA) and included: *cis*-permethrin,
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31 96 *trans*-permethrin, cyfluthrin, and cypermethrin. The physical and chemical properties of the
32
33 97 pyrethroids used in the dosing solutions have been described previously (Wolansky et al. 2006).
34
35 98 Each was provided by its respective manufacturers as follows: permethrin and cypermethrin
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37 99 (FMC Corporation, Philadelphia, PA), deltamethrin and ß-cyfluthrin (Bayer CropScience,
38
39 100 Research Triangle Park, NC), and esfenvalerate (Dupont Crop Protection, Wilmington, DE).
40
41 101 Corn oil was purchased from Fisher Scientific.
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43 102
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45 103 Calibration standards were prepared in reconstituted cleaned extracts of blank tissues and were 1,
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47 104 10, 25, 50, 75 and 100 ng$*$mL^{-1}. Two additional sets of calibration standards were prepared for
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49 105 samples where the calculated concentration of one or more of the pyrethroids extended beyond
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6 106 the original calibration curve. These standards were also prepared in extracted and cleaned
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8 107 tissues and ranged from to 0.25 ng/ml to ng*mL^{-1}, and from 25 ng*mL^{-1} to 1500 ng*mL^{-1}. The
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10 108 surrogate standard ($^{13}C_6$ *trans*-permethrin) was added to all tissues prior to extraction. Internal
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12
13 109 standards ($^{13}C_6$ *cis*-permethrin, $^{13}C_6$ cyfluthrin, and $^{13}C_6$ cypermethrin) were added immediately
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15 110 prior to analysis.
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20 112 **Animals**
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22 113 Male 60 day-old Long Evans rats were purchased from Charles River Laboratories (Raleigh,
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25 114 NC) and allowed to acclimate for a minimum of 4 days in an American Association for the
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27 115 Accreditation of Laboratory Animal Care (AAALAC) approved facility. Rats were housed in
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29 116 pairs in cages (45 cm × 24 cm × 20 cm) lined with heat-treated pine shavings bedding.
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32 117 Temperature, humidity, and light:dark photoperiod were maintained at 21 ± 2°C, 50 ± 10% and
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34 118 12L:12D, respectively. Feed (Purina Rodent Chow 5001, Barnes Supply Co., Durham, NC) and
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37 119 tap water were provided *ad libitum*.
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41 121 **Experimental**
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44 122 The treatment groups in this study consisted of a corn oil control group and two dose mixture
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46 123 levels. Stock mixtures of the pyrethroids at appropriate concentrations were dissolved in corn oil
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49 124 immediately before administration. The preparation was stirred with intermittent heating (max.
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51 125 40-45°C) for at least 15 minutes. All doses (Table 1) were delivered orally in corn oil at 1
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53 126 mL*kg^{-1}. Control animals received corn oil (1 mL*kg^{-1}) only. At 1, 2, 4, 8, or 24 hours post-
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6 127 dosing, the motor activity of each animal was assessed over a one hour period, then the animals
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8 128 were sacrificed (2.5, 3.5, 5.5, 9.5, or 25.5 hours post-dosing) and tissue samples were taken. The
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11 129 number of animals in each group at 1, 2, 4, and 8 hours was: 4 (control), 6 (low dose) and 4 (high
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13 130 dose). At 24 hours there were: 2 controls, 4 low dose, and 4 high dose animals). All animal
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15 131 procedures were approved by the U.S. EPA's National Health and Environmental Effects
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18 132 Research Laboratory's Institutional Animal Care and Use Committee.
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23 134 **Motor Activity**
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25 135 The test used for motor activity was conducted as described by Wolansky *et al.* (2006). Briefly,
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27 136 animals were placed in a series of 16 figure-eight mazes, each with 12 photo-
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30 137 transistor/photodiode pairs. Each beam interruption was recorded as an activity count and
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32 138 captured both horizontal and vertical movement. Testing lasted for 1 hour and total motor
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34 139 activity was the sum of horizontal and vertical counts. Total activity of each test animal was
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37 140 calculated as a percentage of the mean activity of the control animals at the relevant time point.
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41 142 **Tissue Collection and Processing**
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44 143 Anesthesia was induced by CO_2 and cardiac blood was taken via heart puncture during
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46 144 exsanguination. Whole blood was collected in 2 mL aliquots and frozen in a methanol/dry ice
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49 145 bath. Whole brain (seperated at the level of the *foramen magnum*), abdominal subcutaneous fat,
50
51 146 and liver tissues were collected from each animal, post-mortem. These samples were flash
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53 147 frozen in liquid nitrogen and homogenized. All tissue samples were stored at -80°C.
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6 149 The procedures used to extract and purify the pyrethroids were similar to that developed for
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8 150 analysis of deltamethrin in rat tissues (Godin *et al.*, 2010). The mass of brain used for each
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10 151 sample was 350-400 mg and was weighed while frozen. Frozen brain, liver, and fat were
11
12 152 pulverized in a Spex CertiPrep 6850 freezer/mill (Metuchen, NJ) to form a fine homogenous
13
14 153 tissue powder. Blood samples were prepared using the 2 mL aliquots. Prior to extraction, both
15
16 154 brain and blood were placed in a glass culture tube and spiked with $^{13}C_6$-*trans*-permethrin that
17
18 155 served as a surrogate standard. Samples were vortex extracted for 10 minutes with 5 mL
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20 156 acetone:hexane (2:8, V:V) and then centrifuged at 3000 rpm for 10 min. The organic layer was
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22 157 transferred to another culture tube. The extraction was repeated two additional times with 3 mL
23
24 158 acetone:hexane (2:8, V:V). The combined organic extract was dried under nitrogen and
25
26 159 redissolved in 1 mL hexane. The extracts were loaded onto 500 mg silica Solid Phase Extraction
27
28 160 (SPE) columns (Waters, Inc., Milford, MA), rinsed with 5 mL hexane, and eluted with 5 mL of
29
30 161 6% ethyl acetate in hexane. Eluants were dried under nitrogen, then dissolved in 1 mL
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32 162 methanol:water (9:1, V:V). Internal standards were added and the samples transferred to
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34 163 autosampler vials. All samples were stored at -20°C until analysis.
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36 164
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38 165 Fat and liver concentrations were both determined using 250-300 mg samples that were weighed
39
40 166 while still frozen. Prior to extraction, both tissue types were placed in culture tubes and spiked
41
42 167 with $^{13}C_6$-*trans*-permethrin that served as a surrogate standard. Samples were extracted once as
43
44 168 described above and the organic layers were collected. The process was repeated two additional
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46 169 times for the liver with 3 mL acetone:hexane (2:8, V:V) and once with 3 mL acetone:hexane
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6 170 (2:8, V:V) for the fat. The extracts were filtered through polytetrafluoroethylene filters (0.45
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8 171 μm) which were then washed with 3.5 mL acetone:hexane (2:8, V:V). The organic phase was
9
10
11 172 dried under nitrogen and dissolved in 3 mL cyclopentane:ethyl acetate (3:7, V:V). Pyrethroids
12
13 173 were separated from lipids via Gel Permeation Chromatography (GPC) using an OI Analytical
14
15
16 174 Biobead Prep Column J2 Scientific (College Station, TX) and a (3:7, V:V) cyclopentane:ethyl
17
18 175 acetate isocratic mobile phase (5 mL/min). Purified extracts were dried under nitrogen, and
19
20 176 dissolved in 2 mL hexane. Remaining lipids were removed by thrice partitioning the extracts
21
22
23 177 with an equal volume of acetonitrile saturated with hexane. The acetonitrile fractions were
24
25 178 combined, dried under nitrogen, and dissolved in 1 mL methanol:water (9:1, V:V). Internal
26
27 179 standards were added and the samples transferred to autosampler vials. All samples were stored
28
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30 180 at -20°C until analysis.
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32 181
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34 182 **Instrument Analysis**
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37 183 Sample analysis was performed using an AB SCIEX model API 4000™ Liquid
38
39 184 Chromatography-Tandem Mass Spectrometry (LC/MS/MS) system configured with a Turbo Ion
40
41
42 185 Spray (TIS). Tables 1 and 2 of the Supplemental Material list the LC conditions and MS/MS
43
44 186 settings. Under the conditions used, the *cis-trans* isomers of permethrin were separated, but
45
46 187 those isomers were not resolved for cypermethrin or cyfluthrin.
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49 188
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51 189 **Method Validation and Limits of Detection (LOD), and Quantitation (LOQ)**
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53 190 The mean method recoveries of the pyrethroids from each type of tissue were determined using
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56 191 four replicates each of 10 and 75 ng tissue spikes. The LOD and LOQ for each pesticide were
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192 calculated using sixteen replicates of each tissue type spiked with mixture that contained either:
193 250, 500, 750, or 1250 pg of each pyrethroid. The samples were processed and analyzed four
194 separate times over a one-week period. Analyte concentrations were pooled to calculate group
195 standard deviations of the estimated concentrations. Using the standard deviations as the
196 dependent variable and the theoretical concentrations as the independent, least squares regression
197 was used to calculate the intercept. The intercept of the equation was defined as S_0 with the
198 LOD approximated by $3 \times S_0$, and the LOQ approximated by $10 \times S_0$ (Taylor, 1987).
199

200 **Quality Control and Quality Assurance (QC/QA)**

201 A set of matrix-based calibration standards was analyzed immediately before and after each
202 sample set. Quality control procedures included remaking standards when the initial calibration
203 curve data did not fit a first order equation with $r^2 \geq 0.99$. When the slope of the post-run
204 calibration curve differed substantially from the initial, the LC and/or mass spectrometer was
205 cleaned and the samples were re-analyzed. A tissue blank and a mid-level tissue spike sample
206 were each analyzed after each 6 samples in every set.
207

208 The acceptable range for surrogate recovery in the samples was set at 80 - 120%. No surrogate
209 corrections were made to calculated concentrations. When sample surrogate recoveries were
210 outside the acceptance criteria, additional tissue was processed and analyzed. If no tissue was
211 available then the result for that sample was not included in any further analyses. Data below the
212 method LOQ were not used.

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8 214 **Data Analysis**
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11 215 All data were processed and analyzed using SAS/STAT software, version 9.2 (SAS Institute,
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13 216 Cary, NC). Prior to analysis the data were stratified by pyrethroid, tissue, time, and dose (and
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16 217 replicate averaged within rat) and tested to determine data distribution type. The assumption of
17
18 218 normality was supported by Shapiro-Wilk statistics for a majority (>85%) of the distributions,
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20 219 therefore statistical tests for normally distributed data were used.
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23 220
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25 221 Analysis of variance (ANOVA) was used to evaluate method precision and the effect of rats,
26
27 222 replicates, and their interaction on the variability in measured residues within each of the tissue,
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29
30 223 time, and dose groupings. In addition, paired Students t-tests were used to compare replicate
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32 224 measurements, also within tissue, time, and dose groupings.
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37 226 Based on the report by Wolansky *et al.* (2006), peak tissue concentration (brain, blood liver) was
38
39 227 presumed to occur at or before the 2.5 hour time point. The assumption was verified by t-test
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41
42 228 comparisons of concentrations at 2.5 and 3.5 hours and the 2.5 hour time point was used to
43
44 229 compare tissue uptake by pyrethroid and dose group, and, as the first time point in calculation of
45
46 230 the elimination constants. Predicted tissue-blood partition coefficients for all pyrethroids were
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49 231 calculated using the octanol-water partition coefficient based algorithm developed by Poulin and
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51 232 Krishnan (1995).
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234 Elimination constants were estimated using data from hours 2.5 through 9.5 inclusive. Data
235 from the 25.5 hour time point were excluded due to a high percentage of samples with
236 concentrations below the LOQ. First order elimination was assumed and times of sample
237 collection were regressed against transformed residue concentrations (natural logarithmic)
238 grouped by dose and tissue. A generalized linear model (PROC GLM) was used to test each
239 pyrethroid in each tissue for heterogeneity between dose groups and to determine whether each
240 elimination slope was statistically different than zero. Where the slope was not different than
241 zero, that dose group was not used in calculating the pyrethroid half-life. Where the regression
242 slopes of the dose groups were homogeneous and both different than zero, half-lives ($t_{1/2}$) and
243 corresponding confidence bounds of individual pyrethroids in each tissue were estimated using
244 dose-adjusted and dose-pooled data. Then, the heterogeneity of half-lives between individual
245 pyrethroids was evaluated by tissue, also using PROC GLM.
246
247 Relative proportions were calculated for individual pyrethroid concentrations at 2.5 hours. To
248 do this, individual pyrethroids were normalized to equal a percentage of the total pyrethroid load
249 for that tissue and animal. The expected contribution of each pyrethroid equaled the percent
250 pyrethroid of the total pyrethroid dose as listed in Table 1. Tissue-to-blood concentrations were
251 calculated by dividing each tissue concentration (within an animal) by the corresponding blood
252 concentration.
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6 254 Means and standard deviations of motor activity of both dose were calculated at each time point
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8 255 and a four parameter logistic, or sigmoidal E_{max} model (Dmitrienko *et al.*, 2007) was used to
9
10 256 relate variability in motor activity to total pyrethroid brain concentration:
11
12
13
14 $$Y = E_{max} + \frac{(E_{min} - E_{max})}{1 + \left(X/_{EC_{50}}\right)^{h}}$$
15
16
17
18 257 Where Y is the control-normalized response, E_{max} and E_{min} represent the upper and lower bounds
19
20 258 of the response, X is the total pesticide concentration, EC_{50} represents the concentration at which
21
22
23 259 the median response is attained (or inflection point of the curve), and h is the hill coefficient
24
25 260 (slope). Pyrethroids were modeled individually, then by Type (I and II), and finally as a group.
26
27 261 Only data from rats with brain concentrations above the LOQ were included and data from the
28
29
30 262 final time point were not used. Statistical significance was assigned at $p \leq 0.05$.
31
32 263
33
34 264 The hypothetical relative percent contributions of individual pyrethroids in brain tissue in
35
36
37 265 reducing motor activity were estimated as follows:
38
39
40 266 $$RPC_{ij} = \frac{x_{ij} \times RP_i}{\sum_{i=1}^{n} x_{ij} \times RP_i} \times 100$$
41
42
43
44
45 267 Where RPC_{ij} is the relative percent contribution of pyrethroid i to measured response at hour j, x_{ij}
46
47
48 268 is the brain tissue concentration of pyrethroid i at hour j, and RP_i represents the RP of pyrethroid
49
50 269 i (Table 1), assumed to be constant across time. PROC REG was used to calculate the rate of
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52 270 change (in percent contribution) over time for each pesticide. Statistical significance was
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54 271 assigned at $p \leq 0.05$.
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273 **Results**

274 **Method Validation; LOD / LOQ**

275 The pooled percent recoveries (± standard deviation) of all pyrethroids from each tissue type
276 were as follows: blood, 82 ± 7; brain, 92 ± 7; fat, 84 ± 7; and liver, 85 ± 6. The calculated LOQ
277 for all pyrethroids in brain, liver, and fat were less than 3 $ng{*}g^{-1}$ and less than 300 $pg{*}mL^{-1}$ in
278 blood.

279

280 All samples from pyrethroid dosed animals had quantifiable concentrations of all pyrethroids, at
281 both 2.5 and 3.5 hour time points. Measurable concentrations of all pyrethroids were present in
282 all fat samples at all time points. At 5.5 hours, more than 50% of the liver samples (low dose
283 group) had concentrations of *trans*-permethrin (67%) and β-cyfluthrin (83%) that were below the
284 LOQ. At 9.5 hours, all liver concentrations of *trans*-permethrin and β-cyfluthrin, and 66% of
285 deltamethrin concentrations, were below the LOQ. Esfenvalerate in blood (low dose group; 83%
286 ≤ LOQ) at 9.5 hours was the only other time point/analyte where more than 50% of the
287 concentrations were below the LOQ. As stated earlier, data from the 25.5 hour collection were
288 not used due to a lack of quantifiable data from tissues other than fat.

289

290 **Tissue Uptake**

291 At the initial time point (hour 2.5), higher doses generally resulted in higher mean tissue
292 concentrations for each pyrethroid (Table 2). The dose group differences were statistically

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6 293 significant for all pyrethroids in all tissues except: esfenvalerate in brain and cypermethrin,
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8 294 deltamethrin, trans-permethrin and β-cyfluthrin in fat. Comparison of dose-adjusted tissue
9
10 295 concentrations (tissue concentration / dose) at hours 2.5 and 3.5 indicated maximum uptake
11
12
13 296 occurred at or before the 2.5 hour time point.
14
15 297
16
17
18 298 After adjusting for dose, concentrations were not statistically different between the two dose
19
20 299 levels at 2.5 hours for any tissue except blood, where the mean concentrations of all pyrethroids
21
22 300 in the high dose group remained higher than the low dose group. These differences were
23
24
25 301 significant for cypermethrin (p=0.014), deltamethrin (p=0.019), *trans*-permethrin (p=0.002), and
26
27 302 β-cyfluthrin (p=0.025), but not for esfenvalerate (p=0.083), or *cis*-permethrin (p= 0.080). At the
28
29 303 3.5 hour time point, t-tests of dose adjusted concentrations showed that these differences in blood
30
31
32 304 had disappeared.
33
34 305
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36
37 306 At 2.5 hours the most notable difference in relative tissue concentration vs. relative dose
38
39 307 concentration was *trans*-permethrin (31% of total administered) at ≤ 13% of the total pyrethroid
40
41 308 load in all tissues. Using Tables 1 and 2, no consistent relationships appeared between relative
42
43
44 309 tissue concentrations and relative dose concentration, except in the brain. All pyrethroids in
45
46 310 brain, except *cis*-permethrin (48%) and *trans*-permethrin (5%), were consistent with their
47
48 311 relative dosing proportions. However, when *cis* and *trans* isomers are summed, the relative
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51 312 proportion of permethrin is also close to its percentage of 52% in the dosing solution.
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6 314 The theoretical partition coefficients (Poulin and Krishnan, 1995) predicted high tissue-to-blood
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8 315 ratios and little variation for all pyrethroids with coefficients ranging from 21 - 22 (liver-to-
9
10 316 blood), 434 – 438 (fat-to-blood), and 28.1 – 28.4 (brain-to-blood). As seen in Table 3, the tissue-
11
12 317 to-blood ratios for all tissues at the initial time point of this study indicate the partitioning was
13
14 318 much lower and more varied than predicted. Mean brain-to-blood ratios in the high dose group
15
16 319 were all less than those of the low dose group and the ratios for cypermethrin, deltamethrin, and
17
18 320 esfenvalerate were all less than 1. Similar dose dependent differences were observed for all
19
20 321 pyrethroids for the fat-to-blood ratios and, as in the brain, the fat-to-blood ratios of cypermethrin,
21
22 322 deltamethrin, and esfenvalerate were all lower than the permethrins and β-cyfluthrin. The dose
23
24 323 related dependency was repeated in liver-to-blood ratios (initial time point), with the exception
25
26 324 of cypermethrin. As with the other tissues, the liver-to-blood ratio of β-cyfluthrin was nearer the
27
28 325 permethrins than to cypermethrin and deltamethrin, the two most similar type II pyrethroids.
29
30 326 Excepting low dose deltamethrin, the liver-to-blood ratios were all ≥ 1.
31
32 327
33
34 328 **Tissue Elimination**
35
36 329 The calculated elimination constants and relevant statistics for each pyrethroid (by dose group
37
38 330 and tissue) are provided in the Supplemental Material. The half lives, estimated using pooled
39
40 331 high and low group data (where applicable) for blood, brain and liver are located in Table 4.
41
42 332 Half-lives in fat were not calculated because only one of the rate constants (deltamethrin; low
43
44 333 dose, p= 0.04) was statistically different from zero. The half-lives were less than 2 hours in
45
46 334 blood, from 3 to 6.2 hours in brain and 2.3 hours or less in liver. The within-tissue half-lives of
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335 the pyrethroids were not statistically different in blood or liver. In brain, the half-life of β-
336 cyfluthrin was statistically less than all other pyrethroids except *trans*-permethrin. The half-life
337 of cypermethrin was statistically different than the two pyrethroids with the longest half lives;
338 *cis*-permethrin and esfenvalerate, as well as β-cyfluthrin. Finally, the half-life of *trans*-
339 permethrin was statistically different from esfenvalerate.
340
341 **Motor Activity and Brain Concentration of Pyrethroids**
342 Both mixture doses evoked mild clinical signs of pyrethroid toxicity. Restlessness and episodes
343 of non-locomotor behaviors such as scratching, pawing, burrowing and body shakes were
344 observed along the initial 2-3 hours after dosing but there were no signs of high-dose pyrethroid
345 symptoms such as hyper-salivation, whole body tremors and choreoathetosis in any animals.
346 The lack of a full expression of type-specific signs of pyrethroid toxicity was consistent with the
347 mild pyrethroid-specific signs of neurotoxicity observed in a prior pyrethroid mixture study
348 where total doses were higher (Wolansky et al., 2009).
349
350 The results of motor activity (as a percentage of control), for each time period are presented in
351 Figure 1. The time period of peak effect (largest mean decrease) for the low dose group was 1-2
352 hours post dosing. The peak effect for the high dose group occurred during the 2-3 hour interval.
353 After the peak effect, motor activity of both groups increased over the next two time periods,
354 after which no additional increase was apparent.
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356 Excepting esfenvalerate, brain concentrations of individual pyrethroids fit the sigmoidal E_{max}
357 model to predict decreases in motor activity. Similarly, models of Type I and II groups were
358 both statistically significant. Therefore, the final model (Figure 2) used the summed
359 concentration of all pyrethroids. The F-test statistic for the model was significant ($p<0.0001$)
360 with estimated high and low motor activity thresholds of 113 % ($p<0.0001$) and 34 % ($p=0.16$),
361 respectively, of controls. The slope of -2 (between the threshold values) indicated a 2 ng/gm
362 increase in total pyrethroid brain concentration resulted in a 1% decrease in motor activity and
363 the estimated EC_{50} was 217 $ng_{*}g^{-1}$ ($p= 0.0006$).
364

365 Excluding *trans*-permethrin, all regression slopes of estimated relative percent contribution (to
366 total pyrethroid concentration) were statistically significant ($p<0.05$). The slopes for
367 cypermethrin and cyfluthrin were negative while the slopes of esfenvalerate, deltamethrin, and
368 *cis*-permethrin where positive. At 2.5 hours the estimated relative percent contributions were: β-
369 cyfluthrin, 42%; cypermethrin, 21%; deltamethrin, 13%; esfenvalerate, 13%; *cis*-permethrin, 8%;
370 *trans*-permethrin 1%. At 9.5 hours these values had changed to: β-cyfluthrin, 23%;
371 cypermethrin, 17%; deltamethrin, 17%; esfenvalerate, 26%; *cis*-permethrin, 18%; *trans*-
372 permethrin 1%.
373

374 **Discussion**

375 **Implications of Study Design**

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6 376 The use of low dose, empirically based, pyrethroid mixtures to estimate toxicokinetic parameters
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8 377 is a significant advance in connecting exposure science with toxicology. Although simplified in
9
10 378 its use of a single exposure pathway and single dose design, this study incorporated the concept
11
12
13 379 of environmentally-relevant cumulative dose in evaluating tissue uptake and elimination. Use of
14
15 380 a mixture provided direct comparative data for uptake and elimination of each pyrethroid in each
16
17 381 tissue. Simultaneous dosing with five pyrethroids that included representatives of Types I and II
18
19
20 382 helped to address whether non target site kinetic differences were important correlates of motor
21
22 383 activity. Although not assessed directly, potential interactions between the pyrethroids were
23
24 384 inherent in this study design and reflect interactions expected from actual exposures.
25
26
27 385
28
29 386 **Kinetics**
30
31 387 **Liver**
32
33
34 388 Relative to dose, liver concentrations of *trans*-permethrin at 2.5 hours were much lower than *cis*-
35
36 389 permethrin and the other pyrethroids (Tables 1 and 2). This result was unexpected due to the
37
38
39 390 similarity of all pyrethroid half lives in both blood and liver (Table 4), and the narrow range of
40
41 391 predicted pyrethroid liver-to-blood partition coefficients (21 – 22). It is possible that the *trans*-
42
43
44 392 permethrin concentrations resulted from increased binding of *trans*-permethrin by hepatic or
45
46 393 circulatory proteins, but literature reports suggest that covalent binding rates of pyrethroids by
47
48 394 hepatic proteins are generally low. An *in vivo* study of (Hoellinger *et al*., 1983) found that less
49
50
51 395 than 6% each of the type I pyrethroids cismethrin and bioresmethrin were covalently bound to rat
52
53 396 liver proteins. In addition, Catinot *et al*. (1989) found the covalent binding in homogenates of rat
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56 397 liver to be less than 10% for the type II pyrethroids deltamethrin and cypermethrin.
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6 398 Unfortunately, a literature review revealed no reports of *cis* or *trans*-permethrin hepatic protein
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8 399 binding.
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13 401 The lower *trans*-permethrin concentrations may have resulted from significant levels of intestinal
14
15 402 metabolism, or less absorption. Crow *et al.* (2007) and Nakamura *et al.* (2007) also reported that
16
17 403 intestinal and hepatic cytosol carboxylesterases could cleave the ester linkage of *trans*-
18
19
20 404 permethrin but not deltamethrin or *cis*-permethrin. However, Nakamura *et al.* (2007) reported
21
22 405 rat intestinal hydrolysis rates to be only 33% of the liver and Crow *et al*. (2007) estimated that
23
24 406 intestinal hydrolysis accounted for only 2.5% of the total hydrolytic activity for *trans*-permethrin
25
26
27 407 in the rat. The dose adjusted concentration (dose groups pooled) of β-cyfluthrin in the liver at
28
29 408 2.5 hours was lower than that of *cis*-permethrin and cypermethrin. This was unexpected because
30
31
32 409 β-cyfluthrin is a type II pyrethroid, which differs from cypermethrin by addition of fluorine at the
33
34 410 4 position of the alcohol moiety and enrichment of 2 pairs of diasteromers, specifically, (S)α,
35
36
37 411 1(R)-*cis*- + (R)α, 1(S)-*cis*-; (S)α, 1(R)-*trans*- + (R)α, 1(S)-*trans*-. Unfortunately, there appear to
38
39 412 be no published reports describing cyfluthrin toxicokinetics.
40
41 413
42
43
44 414 Single pyrethroid *in vitro* studies have shown pyrethroid specific differences in rates of
45
46 415 metabolism and the importance of different pathways. In general, the predicted order of
47
48
49 416 clearance rates for pyrethroids (Soderlund *et al*., 1977) in mixed esterase/oxidase systems is:
50
51 417 *trans* esters (unsubstituted at primary alcohol) > *cis* esters (unsubstituted at primary alcohol) >
52
53 418 *trans* esters (substituted at α carbonyl) > *cis* esters (substituted at α carbonyl) and it is not clear
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419 why the liver half lives of all pyrethroids in the current study were approximately equal. As
420 Soderlund used mouse microsomal preparations it is possible that the disparity is due to *in vivo*
421 vs. *in vitro* or species related differences. Literature reports of *in vivo* or *in vitro* metabolic rates
422 determined using a pyrethroid mixture are largely absent. However, there is some evidence of
423 interaction between the *cis*/*trans* isomers of permethrin. Scollon *et al.* (2009) demonstrated an *in*
424 *vitro cis*/*trans* interaction between permethrin isomers whereby the clearance of the *trans* isomer
425 was slowed significantly in the presence of the *cis* isomer while elimination of the *cis* isomer was
426 slowed slightly.
427
428 Clearly, more in vitro work evaluating interactions of pyrethroids and their *cis*/*trans* isomerism
429 in esterase/oxidative mediated metabolic systems would be useful. These data suggest that
430 intestinal metabolism may explain some of the differences between *cis*- and *trans*-permethrin
431 toxicokinetics, but other mechanisms, such as differences in absorption, may also play a role.
432 Further research needs to be performed to determine whether the loss of the *trans*-isomer extends
433 to cypermethrin and β-cyfluthrin as both are also *cis*/*trans* mixtures.
434
435 **Blood**
436 The transient dose-dependent concentration of the pyrethroids in the blood at 2.5 hour indicates
437 non-linear absorption kinetics. The non-linearity may be attributed to absorption rather than
438 metabolism because of the consistency between the estimated elimination half-lives of the
439 pyrethroids (Table 4) in blood. The pyrethroids in the high dose group may have been absorbed
440 more efficiently because they were more concentrated in the corn oil vehicle. For example,

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441 Wolansky *et al*. (2007) found that reducing the volume of the corn oil vehicle five-fold,
442 increased the potency of orally administered bifenthrin by a factor of two. Further, in the current
443 study, all pyrethroids showed this dose related difference regardless of their relative
444 concentration in the mixture, suggesting the effect was related to the total pyrethroid
445 concentration, rather than the concentration of the individual pesticides. Although Godin *et al.*
446 (2010) examined the bioavailability of deltamethrin and found it to be dose independent (0.3 and
447 3 mg/kg), the total pyrethroid doses in this study (Table 3) were higher than the doses of
448 deltamethrin in the cited study.
449
450 When the two dose groups were pooled at 2.5 hours, the liver-to-blood ratio of deltamethrin
451 (0.88 ± 0.24) was similar to the 1:1 ratio reported by Mirfazaelian *et al*. (2006), but higher than
452 the 2:7 ratio seen by Godin *et al*. (2010). Published reports with this type of *in vivo* data for the
453 other pyrethroids are lacking. In the current study, the liver-to-blood ratios of all pyrethroids
454 were 1:1 or greater and t ½'s of all pyrethroids in blood were not different than in liver. In
455 addition, the liver-to-blood ratio of deltamethrin was significantly lower ($p \leq 0.05$) than all other
456 pyrethroids except cypermethrin. As stated earlier, differences in octanol-water based partition
457 coefficients estimations are very small and do not explain differences in liver-to-blood ratios.
458 Alternatively, differences in blood protein binding may provide an explanation, but studies of the
459 capacity of serum proteins to bind permethrin (Abu-Qare and Abou-Donia 2002), cismethrin,
460 and bioresmethrin (Hoellinger *et al*. 1985) suggests that this pathway is not significant.
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6 462 **Fat**
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8 463 Fat functioned effectively as a sink for the pyrethroids in this study as all were more
9
10 464 concentrated in fat than other tissues. The rapid uptake and lack of elimination in fat throughout
11
12 465 the study time course was expected since pyrethroids are lipophilic and lipases are not thought to
13
14 466 be important in the metabolism of pyrethroids (Crow *et al.*, 2007). The results are consistent
15
16 467 with observed slow elimination rates *in vivo* studies of deltamethrin (Godin *et al.*, 2010;
17
18 468 Mirfazaelian *et al.*, 2006). Assuming fat comprises 7% of total rat body mass (Schoeffner *et al.*,
19
20 469 1999), the percent of each pyrethroid in fat was less than 2% of its administered dose. The
21
22 470 uptake by adipose tissue is low compared to other lipophillic chemicals such as dioxin (Diliberto
23
24 471 *et al.*, 1996). Because the octanol-water partition coefficients of pyrethroids and dioxin are
25
26 472 comparable, the difference in adipose tissue distribution is likely due to a higher rate of
27
28 473 pyrethroid metabolism in other tissues.
29
30 474
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32 475 Excepting low dose esfenvalerate, concentrations in fat did not increase during the study
33
34 476 (Supplemental Table 3). Therefore, the data did not support significant redistribution of the
35
36 477 pyrethroids from other tissues to the fat. In addition, the long half-life of the pyrethroids in the
37
38 478 fat and rapid metabolism in liver and blood precluded adipose tissue from being a secondary
39
40 479 source for measurable redistribution to other tissues.
41
42 480
43
44 481 **Brain Kinetics and Motor Activity**
45
46 482 High-low dose related differences in blood concentrations at 2.5 hours were not reflected in the
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48 483 brain. In addition, all brain-to-blood ratios were much lower than predicted by their partition
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6 484 coefficients, suggesting a limitation in crossing the blood brain barrier that affected all study
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8 485 pyrethroids. Brain-to-blood ratios did not segregate into Type I and Type II pyrethroids (Table
9
10 486 3), nor did they appear to be a function of the absolute concentration in the blood or differences
11
12 487 in brain elimination rates. Ultimately crossing the blood-brain barrier by each pyrethroid is
13
14 488 likely determined by its tertiary structure and a more sophisticated analysis of pyrethroid
15
16 489 structure activity relationships would be useful.
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18 490
19
20 491 The observed differences in $t_{1/2}$ of the pyrethroids in the brain pyrethroid did not sort according
21
22 492 to the rates predicted from oxidative and hydrolytic activity in liver or serum. Interestingly,
23
24 493 Ghiasuddin and Soderlund (1984) found the specificity of mouse brain esterases were different
25
26 494 than the mouse liver esterases and the brain esterases demonstrated activity toward *trans*-
27
28 495 permethrin and fenvalerate, but were relatively inactive in the hydrolysis of *cis*-permethrin or
29
30 496 deltamethrin. Potentially, this may also occur in the rat.
31
32 497
33
34 498 An important finding of this study was the similarity between the relative proportion of each
35
36 499 pyrethroid in the brain and its percentage in the dosing mixture (after summing *cis*- and *trans*-
37
38 500 permethrin isomers) at the 2.5 hour time point. Therefore, administered dose predicted relative
39
40 501 brain concentrations at a time point near the peak effects of individual pyrethroids on motor
41
42 502 activity as noted by Wolansky *et al.* (2009). This may simplify efforts to model low dose
43
44 503 cumulative risk from these pyrethroids since it appears metabolic variations in other tissues were
45
46 504 offset by differences in partitioning from blood to brain. The similarity of the relative
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6 505 proportions of the pyrethroids in the brain and dosing solution implies that differences in RP of
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8 506 each pyrethroid are not a function of toxicokinetic differences. Rather, their relative impact on
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11 507 motor activity is likely dependent on specific interactions with ion channels.
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16 509 The decreases in motor activity of 34% (low dose, 1.5x ED_{30}) and 67% (high dose, 3.7x ED_{30}) at
17
18 510 the times of peak effect (1-2 hours low dose group, 2-3 hours high dose group) agree with the
19
20 511 decrease of approximately 40% and 60% predicted by Wolansky *et al.* (2009) and therefore
21
22
23 512 support those researchers pyrethroid dose additive effects model. The occurrence of greatest
24
25 513 mean decrease in motor activity at 1-3 hours is consistent with the time to peak effect for the
26
27 514 individual pyrethroids of 1.5 to 2.0 hours also reported by Wolansky *et al.* (2009). That studies'
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29
30 515 finding of a return to normal motor activity function several hours post dosing, also occurred in
31
32 516 this study (Figure 2). In addition, the minimum dose threshold, linear dose response range, and
33
34 517 asymptotic nature of the maximum response in the Wolansky model were apparent in this studies
35
36
37 518 4 parameter model of motor activity and pyrethroid brain concentration (Figure 2). The brain
38
39 519 concentrations of the pyrethroids in this study provide the toxicokinetic data underlying the dose
40
41
42 520 additive pyrethroid effects model and assist the interpretation of that model by showing the
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44 521 relationship between response and the concentration of this mixture of pyrethroids in the brain.
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46 522 Further, the fit of the 4 parameter model ($p < 0.001$) and the overlap of the data from the two
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49 523 dose groups at 4 time points indicates that the pyrethroid brain concentration is the important
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51 524 determinate of motor activity and that dose and time predict brain concentration and therefore
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53 525 effect. This supports research by Scollon *et al.* (2011) who found a similar relationship between
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56 526 bifenthrin dose, brain concentration, and motor activity.
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8 528 Although the contribution of individual pyrethroids to loss of motor activity could not be directly
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11 529 measured, the hypothetical relative contributions suggest that β-cyfluthrin would be the greatest
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13 530 contributor to loss of motor activity at 2.5 hours, and *cis*/*trans*-permethrin the least. At 9.5 hours
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15 531 individual pyrethroids would contribute similarly to total toxicity. It is important to note that
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18 532 these estimates should be interpreted cautiously as Wolansky *et al.* (2006) determined RP's in
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20 533 single chemical studies using multiple doses at the time of peak effect. Additional data on RP
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22 534 from single chemical, time-series studies would provide insight into the validity of combining
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25 535 RP and tissue concentration across time in this model.
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30 537 **Conclusions**
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32 538 Environmentally relevant dosing schemes may be used as a conceptual model in future studies of
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34 539 chemical mixtures. In this study it added a practical component to a toxicokinetic study that
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37 540 would be useful in cumulative risk assessments.
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41 542 Distribution of the pyrethroids to all tissues was rapid with maximum concentrations likely at, or
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44 543 before, the 2.5 hour time point. Excepting blood at 2.5 hours, relative tissue concentrations were
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46 544 dose independent. In liver, blood, and fat there was no apparent relationship between uptake and
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49 545 pyrethroid relative dose concentrations, lipid solubility, or structural groupings. The most notable
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51 546 results were the very low proportionate tissue concentrations of *trans*-permethrin.
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6 548 Initial concentrations of cypermethrin, deltamethrin, β-cyfluthrin, and esfenvalerate in brain
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8 549 reflected their relative proportion in the dosing solution. When concentrations *cis* and *trans*-
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10 550 permethrin were summed, the relative brain permethrin concentration also reflected dose.
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15 552 The similarity of the elimination half-lives of individual pyrethroids in blood and liver was
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17 553 unexpected considering *in vitro* differences of previous studies. In brain, half-life differences
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19 554 between the slowest and most rapidly cleared pyrethroids were unrelated to relative dose
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21 555 concentration or obvious differences in chemical structure.
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25 557 Brain concentration was predicted by dose and time, and pyrethroid brain concentration
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27 558 predicted motor activity. Relative uptake and elimination of the pyrethroids in brain did not
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29 559 correspond with the Type I and Type II groupings or the RP's noted in previous studies.
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31 560 Therefore, differences in pyrethroid kinetics are insufficient to explain behavioral responses
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33 561 observed in previous studies.
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35 562
36
37 563 **Supplementary Data:** The supplementary material consists of four tables. Table 1 lists the
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39 564 chromatograph and mass spectrometer settings used for all analysis while analyte specific mass
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41 565 spectrometer settings are provided in Table 2. Estimated pyrethroid elimination constants and
42
43 566 associated test statistics for each dose group and each tissue are presented in Table 3.
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53 568 **Funding:** This work was supported by The United States Environmental Protection Agency
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55 569 through its Office of Research and Development who funded and managed the research
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570 described here. It has been subjected to Agency administrative review and approved for
571 publication. This does not signify that the contents necessarily reflect the views and policies of
572 the Agency, nor does mention of trade names or commercial products constitute endorsement or
573 recommendation for use.
574

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7 589 **Table 1.** Concentration of pyrethroids in low and high dose mixtures.
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Pyrethroid	Type[a]	Low Dose (mg⁎kg^{-1})	High Dose (mg/⁎kg^{-1})	% of Total Dose	Relative Potency[b]
cypermethrin	II	3.2	7.9	29	0.235
deltamethrin	II	0.4	0.9	3	1.000
esfenvalerate	II	0.3	0.7	3	2.092
cis-permethrin	I	2.3	5.7	21	0.059[c]
trans-permethrin	I	3.5	8.6	31	
β-cyfluthrin	II	1.5	3.5	13	1.136
Total Dose		11.2	27.4		

31 590 [a] Based on presence or absence of a cyano group at the α-carbon of the alcohol moiety, and the
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33 591 high dose acute physiological effects.
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36 592 [b] Potency based on ED_{30} for effect on motor activity relative to deltamethrin as the index
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38 593 pyrethroid (Wolansky *et al.*, 2006).
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41 594 [c] Relative potencies of *cis*/*trans*-permethrin isomers were not determined independently.
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55 605 **Table 2.** Tissue concentration by dose group at 2.5 hours after dosing.
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			Mean tissue concentration ± standard error (blood ng*mL^{-1}; brain, fat, liver ng*g^{-1})					
tissue	**dose group**	**n**	**cypermethrin**	**deltamethrin**	**esfenvalerate**	***cis*-permethrin**	***trans*-permethrin**	**β-cyfluthrin**
blood	Low	4	141[a] ± 21	25[a] ± 4	12[a] ± 2	52[a] ± 9	5[a] ± 2	15[a] ± 3
	High	4	647 ± 77	115 ± 14	52 ± 12	196 ± 19	46 ± 4	87 ± 19
brain	Low	6	68[a] ± 9	10[a] ± 1	7 ± 1	122[a] ± 16	11[a] ± 2	28[a] ± 4
	High	3	143 ± 15	19 ± 4	10 ± 3	230 ± 31	32 ± 6	58 ± 9
fat	Low	6	565 ± 112	83 ± 11	72[a] ± 9	512[a] ± 77	216 ± 37	233 ± 48
	High	3	1076 ± 239	158 ± 51	146 ± 34	908 ± 165	406 ± 93	466 ± 111
liver	Low	6	140[a] ± 27	26[a] ± 4	25[a] ± 4	95[a] ± 15	18[a] ± 6	38[a] ± 9
	High	3	400 ± 26	71 ± 5	65 ± 5	219 ± 10	64 ± 18	139 ± 14

606 [a] Mean concentration statistically different ($p \leq 0.05$) than high dose concentration

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622 **Table 3.** Tissue-to-blood pesticide concentration ratios

Dose Group	hour	cypermethrin	deltamethrin	esfenvalerate	*cis*-permethrin	*trans*-permethrin	β-cyfluthrin
		Mean Brain-to-Blood Concentration Ratio (± standard error of the mean)					
Low	2.5	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	2.6 ± 0.4	3.2 ± 1.1	2.1 ± 0.4
High	2.5	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	1.2 ± 0.6	0.7 ± 1	0.7 ± 0.4
All	3.5	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.4	3.3 ± 1.3	3 ± 1.4	2.4 ± 0.9
	5.5	1.7 ± 0.2	3.4 ± 1.0	4.6 ± 0.9	16.3 ± 5	19.5 ± 12.3	7.4 ± 1.5
	9.5	2.2 ± 0.6	4.3 ± 1.6	3.4 ± 1.2	49.2 ± 15.2	15.8 ± 2.7	7.4 ± 1.9
	25.5	3.4 ± 1.5	NA[1]	NA	26.5 ± 12.3	NA	NA
		Mean Fat-to-blood Concentration Ratio (± standard error of the mean)					
Low	2.5	4 ± 1	4 ± 0	6 ± 1	11 ± 2	58 ± 20	17 ± 4
High	2.5	2 ± 0	1 ± 0	3 ± 0	5 ± 1	9 ± 2	6 ± 1
All	3.5	6 ± 2	8 ± 4	16 ± 9	9 ± 1	38 ± 11	36 ± 18
	5.5	33 ± 7	44 ± 10	69 ± 13	136 ± 58	372 ± 120	187 ± 36
	9.5	134 ± 41	142 ± 30	119 ± 42	1056 ± 424	1476 ± 507	901 ± 232
	25.5	1963 ± 868	462 ± 63	NA	3153 ± 975	1895 ± 729	6512 ± 2683
		Mean Liver-to-Blood Concentration Ratio (± standard error of the mean)					
Low	2.5	1.0 ± 0.2	1.0 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	2.8 ± 0.7	2.1 ± 0.3
High	2.5	1.6 ± 0.1	0.6 ± 0	1.3 ± 0.2	1.1 ± 0.1	1.4 ± 0.3	1.7 ± 0.2
All	3.5	1.4 ± 0.1	0.7 ± 0	2.3 ± 0.7	1.3 ± 0.1	1.9 ± 0.4	7.6 ± 5.6
	5.5	1.2 ± 0.1	1 ± 0.1	3.4 ± 1.5	2.6 ± 0.7	9.4 ± 5	2.2 ± 0.8
	9.5	2.2 ± 0.1	4.3 ± 0.3	3.4 ± 0.7	49.2 ± 0.7	15.8 ± 2	7.4 ± 0.8
	25.5	0.4 ± 0.1	NA	NA	NA	NA	NA

1 insufficient data were available to calculate a ratio.

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636 **Table 4.** Estimated pyrethroid half-lives ($t_{1/2}$) in rat tissues.

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Tissue	Pyrethroid	$t_{1/2}$ (hours)	LCL[a] (hours)	UCL[a] (hours)
Blood	cypermethrin	1.5	1.2	2.1
	deltamethrin	1.5	1.2	2.0
	esfenvalerate	1.6	1.2	2.5
	cis-permethrin	1.3	1.1	1.8
	trans-permethrin	1.2	0.9	1.8
	β-cyfluthrin	1.3	1.0	1.8
Brain	cypermethrin	2.7[b]	2.3	3.4
	Deltamethrin	3.9[b]	2.8	6.3
	esfenvalerate	6.2[bcd]	3.4	41.1
	cis-permethrin	5.5[bcd]	4.3	7.7
	trans-permethrin	2.7	2.1	3.9
	β-cyfluthrin	2.0[c]	1.7	2.4
Liver	cypermethrin	1.6	1.3	2.2
	deltamethrin	1.9	1.4	2.8
	esfenvalerate	1.8	1.4	2.5
	cis-permethrin	1.6	1.3	2.1
	trans-permethrin	2.3	1.4	6.1
	β-cyfluthrin	1.3	1.0	2.1

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638 [a] Estimated lower (LCL) and upper (UCL) 95% confidence limits of the mean $t_{1/2}$.

639 [b] Half-life is statistically different ($p \leq 0.05$) than β-cyfluthrin

640 [c] Half-life is statistically different ($p \leq 0.05$) than cypermethrin

641 [d] Half-life is statistically different ($p \leq 0.05$) than *trans*-permethrin

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645 Figure 1. Time course of changes motor activity after an oral dose of a pyrethroid mixture.
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647 Figure 2. Four parameter model of brain pyrethroid concentration and motor activity.
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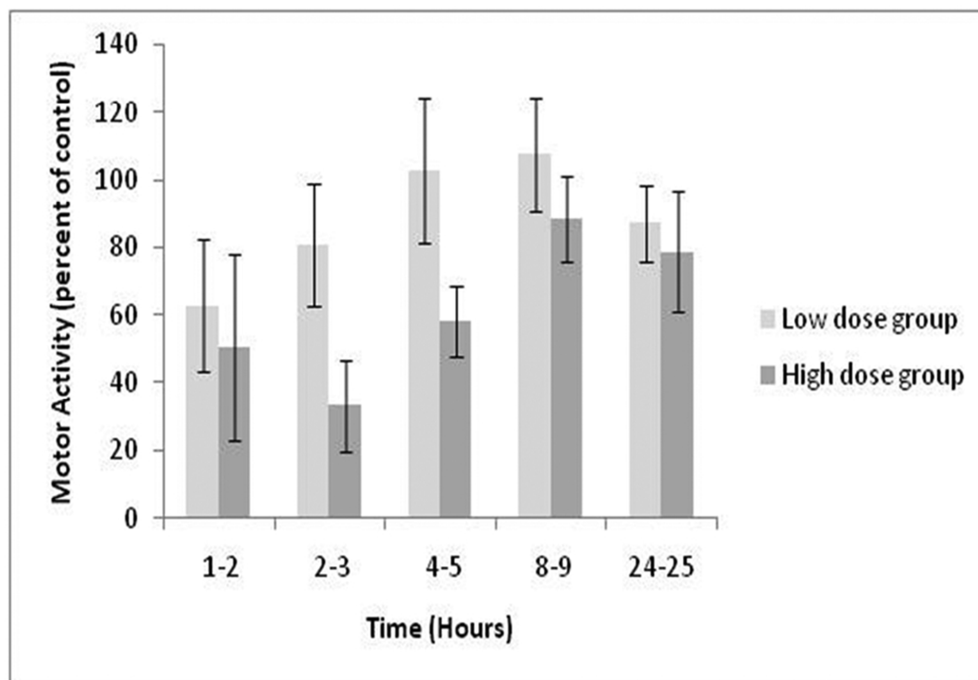

Figure 1. Time course of changes motor activity after an oral dose of a pyrethroid mixture.
88x61mm (300 x 300 DPI)

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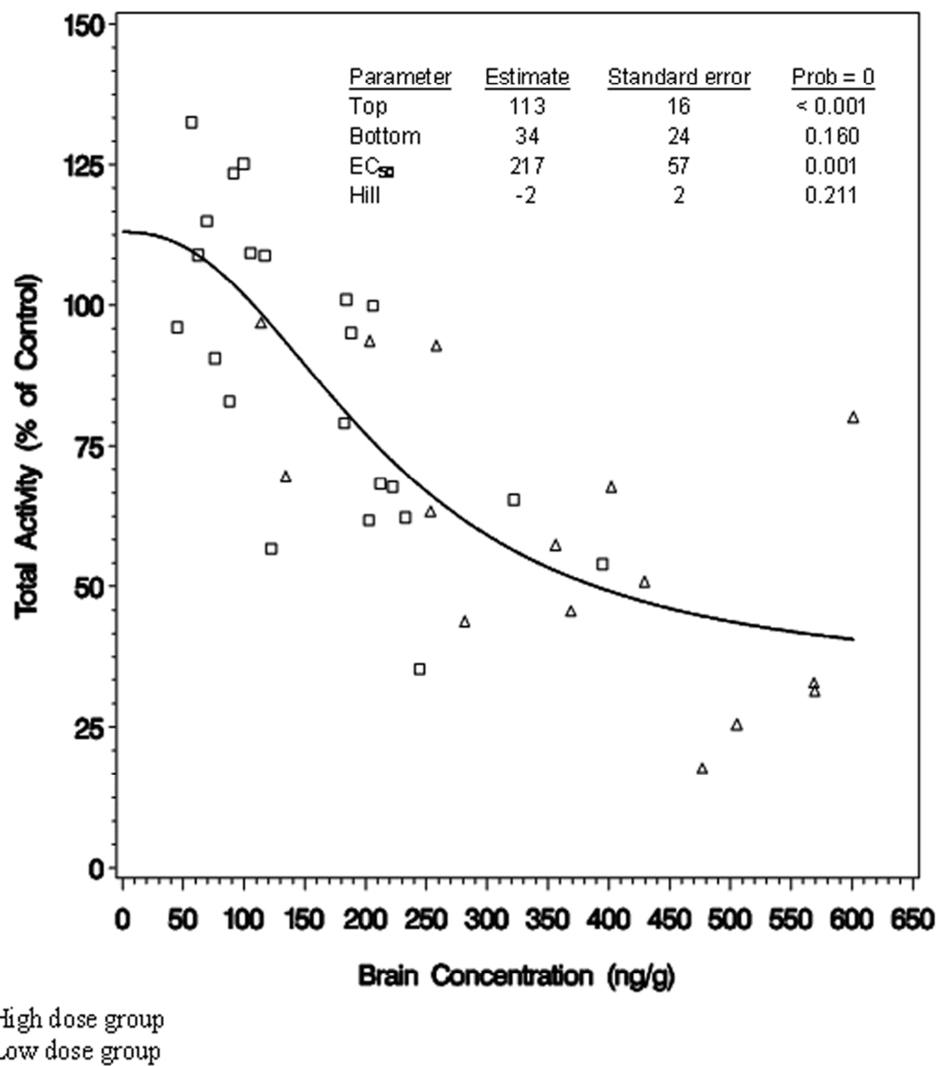

Figure 2. Four parameter model of brain pyrethroid concentration and motor activity.
88x96mm (300 x 300 DPI)

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