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Influence of dosing volume on the neurotoxicity of bifenthrin ☆

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Abstract

Pyrethroids are pesticides with high insecticidal activity and relatively low potency in mammals. The influence of dosing volume on the neurobehavioral syndrome following oral acute exposure to the Type-I pyrethroid insecticide bifenthrin in corn oil was evaluated in adult male Long Evans rats. We tested bifenthrin effects at 1 and 5 ml/kg, two commonly used dose volumes in toxicological studies. Two testing times (4 and 7 h) were used in motor activity and functional observational battery (FOB) assessments. Four to eight doses were examined at either dosing condition (up to 20 or 26 mg/kg, at 1 and 5 ml/kg, respectively). Acute oral bifenthrin exposure produced toxic signs typical of Type I pyrethroids, with dose-related increases in fine tremor, decreased motor activity and grip strength, and increased pawing, head shaking, click response, and body temperature. Bifenthrin effects on motor activity and pyrethroid-specific clinical signs were ~2-fold more potent at 1 ml/kg than 5 ml/kg. This difference was clearly evident at 4 h and slightly attenuated at 7 h post-dosing. Benchmark dose (BMD) modeling estimated similar 2-fold potency differences in motor activity and pyrethroid-specific FOB data. These findings demonstrate that dose volume, in studies using corn oil as the vehicle influences bifenthrin potency. Further, these data suggest that inconsistent estimates of pyrethroid potency between laboratories are at least partially due to differences in dosing volume.
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Keywords: Pyrethroids; Neurotoxicity; Bifenthrin; Motor activity; Functional observational battery; Vehicle effects

1. Introduction

Pyrethroids are insecticides used for both indoor and outdoor applications [3,11,20,47]. Pyrethroids are neurotoxic [39,45], with a primary site-of-action on voltage-sensitive sodium channels on neuronal axons [31]. Acute pyrethroid exposure causes syndromes of toxicity in rats and mice that are generally characterized into two classes: those pyrethroids inducing whole body tremor (i.e., Type I or T compounds) and those evoking choreoathetosis and salivation (i.e., Type II or CS compounds). A few compounds that do not fit this dual classification have been proposed to represent a third subgroup that induces both tremor and salivation [13,22,48].

Pyrethroid toxicity in rodents is sensitive to various experimental conditions, including dosing vehicle [6,52], route of exposure [6,34], stereoisomer composition [14,45,49], and commercial formulation [1,23,28,50,53]. Previous work from our laboratory demonstrated potency differences for deltamethrin due to differences in vehicle or route of administration. The ED50 (50% reduction in motor activity) was shifted from 5.1 mg/kg to >1000 mg/kg when the vehicle was changed from corn oil to methylcellulose [6]. In addition, the ED50 was shifted from 5.1 to 38.9 when the route was changed from oral gavage to intraperitoneal, using a corn oil vehicle [6]. Nevertheless, there are knowledge gaps concerning the impact of relevant exposure conditions on pyrethroid toxicity.

Dose-effect data and relative potency factors for the effects of eleven pyrethroids on motor activity were recently generated,

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with all pyrethroids producing dose-dependent decreases in activity [55]. Potency estimates for many of these compounds were similar to those generated previously in the same laboratory [6–8]. In contrast, these estimates were much lower than the reported no-observed effect levels from neurobehavioral studies conducted according to US EPA neurotoxicity guidelines [32] as reported in [2,32,43]. These discrepancies were not due to differences in chemical purity: the chemicals used in Wolansky et al. [55] and Nemec [32] were either from the same manufacturing batch or of similar isomeric composition. One discrepancy between the two studies was the dosing volume. Both laboratories used acute gavage as the route of exposure, with corn oil as the solvent. However, Wolansky et al. [55] used 1 ml corn oil/kg as the vehicle dose volume, and Nemec [32] used 5 ml corn oil/kg. Vehicle volume has been reported to influence the potency of DDT's effects on neurobehavioral outcomes [25]. In addition, use of corn oil vehicle delays the absorption of lipophic chemicals (e.g., carbon tetrachloride) relative to the neat compound [21,54].

The current work tested the hypothesis that increasing the dosing volume of corn oil decreases the potency of bifenthrin. Bifenthrin was used as an exemplar of a Type I pyrethroid that causes fine tremor, whole body tremor, uncoordinated movements, ataxia, and decreased motor activity [15,40,55]. The ED30 (dose producing a 30% decrease in motor activity) for bifenthrin in rats when given in a dose volume of 1 ml/kg is 3.2 mg/kg [55]. Nemec [32] reports a LOEL (lowest dose associated with an effect) of 40 mg/kg for clinical signs when bifenthrin is given in a dose volume of 5 ml corn oil/kg. While ED30's and LOELs are not completely compatible, nonetheless, this evidence suggests that using higher dose volumes attenuates the neurotoxic effects of bifenthrin. The hypothesis was tested using the functional observational battery (FOB) [26] to characterize the clinical signs of bifenthrin toxicity and assessments of spontaneous activity in the figure-eight maze [35,41] at two dose volumes (i.e., 1 and 5 ml/kg) and two relevant testing times during the peak intensity of the syndrome. Motor activity is a reliable and consistent marker of pyrethroid toxicity in rodents [6–8,16,17,24,26,38,55]. In addition, the FOB has been used previously to characterize pyrethroid type-specific clinical profiles [26], and to detect dose-volume related changes in potency of DDT [25], an organochlorine pesticide having the same primary target site as pyrethroids [30]. The results of this work show that increasing dose volume delays the onset of toxicity and decreases the potency of bifenthrin.

2. Methods

2.1. Subjects

Male Long Evans rats (Charles River Laboratories, Inc., Wilmington, MA) were obtained at 55–58 days of age, and housed two per cage in standard polycarbonate hanging cages (45 cm × 24 cm × 20 cm) containing heat sterilized pine shavings (Northeastern Products, Inc., Warrensburg, NY). All animals were given a 5–9 day acclimation period and were maintained on a 12:12 h photoperiod (L:*D*, 0600:*1800*). Food (Purina 5001 Lab Chow) and water were provided *ad libitum*. Tap water (Durham, NC water) was filtered through sand, then activated charcoal, and finally re-chlorinated to 4–5 ppm Cl^- before use in the animal facility. Colony rooms were maintained at 22.0±2.0 °C and relative humidity at 50±10%. The facility is approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All experimental protocols were approved in advance by the US EPA's National Health and Environmental Effects Research Laboratory Animal Care and Use Committee.

2.2. Chemicals

Technical grade (89% pure) bifenthrin (IUPAC: 2-methyl-3-phenyl-phenyl)methyl 3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethyl-cyclopropane-1-carboxylate; 99%+ (*Z*)-(1*R*)-*cis* isomer) was kindly supplied by its manufacturer (FMC Corp., Philadelphia, USA). Note that this pyrethroid was from a lot with similar physical and chemical properties as that used in the manufacturer-sponsored studies [45] and the same technical grade that was used by Nemec [32]. Doses were calculated based on percent active ingredient in the technical product. Fresh bifenthrin stock and dosing solutions were prepared daily by dissolving in corn oil (Sigma, Co., USA). The dosing solutions were intermittently stirred and gently heated (40–50 °C) to ensure complete solubilization in the vehicle. Dosing solutions were used at room temperature.

2.3. Animal treatment

Bifenthrin was administered by oral gavage using 18 gauge intubation needles (Popper and Sons, Inc., New Hyde Park, NY) in two different dose volumes (see Table 1). Dosages evoking excessive toxicity (i.e., leading to prolonged hyperexcitation and whole body tremors, or mortality) were not used to ensure estimations of pyrethroid-specific alterations and not functional depression due to near-lethal intoxication. Prior to dosing, animals were moved from the colony to an isolated room within the testing laboratories where treatments were administered after one-hour acclimation. Each experiment was divided into two or more blocks as appropriate. Vehicle-intubated controls were included in each block. Order of testing and time of day were counter-balanced across treatment groups. New, naïve, independent groups of rats were used for each experiment. All animals were observed before and after testing runs for signs of excessive toxicity. All testing was conducted between 0900 and 1700 h.

2.4. Time-course assessment (Experiment 1)

Preliminary work was conducted to define the time course of the neurobehavioral syndrome evoked by bifenthrin. Pilot studies (data not shown) were used to determine functional equivalent doses of bifenthrin in the two different vehicle volumes. Animals were tested in figure-8 mazes (see below) for 1 h following doses of 12 mg/kg in 1 ml/kg and 20 mg/kg in 5 ml/kg. Separate groups of animals were tested at times from

Table 1
Dosing and testing conditions, and endpoints in each experiment

Exp.	Behavioral domain	Behavioral endpoints	Dose levels (mg/kg)	Dose volume (ml/kg)	Testing time (h)	*N* per group
1	Neuromotor	Figure 8 maze activity	12	1	1, 2, 3, 4, 7	6–12
			20	5	1, 3, 5, 7	
2			0.1, 1, 2, 4, 6, 8, 12, 16	1	4	8–20 [a]
			0.1, 1, 6, 9, 12, 20	5		
			0.01, 0.1, 1, 2, 4, 6, 8, 12	1	7	
			1, 6, 9, 12, 14, 20	5		
3	Pyrethroid	Tremors, click response, pawing, head shaking, body temperature	1, 6, 12, 20	1	3.5–4 and 6.5–7 (FOB)	
	Neuromotor	Gait score, grip strength Figure 8 maze activity	6, 12, 20, 26	5	4 and 7 (motor activity)	10 [b]
	Reactivity	Handling reactivity, tail pinch response, arousal				

[a] $n=4$ at 2 and16 mg/kg using 1 ml/kg (4 h time point).
[b] $n=9$ in motor activity assessment at 5 ml/kg, 26 mg/kg (4 h time point).

one to 7 h post-dosing (see Table 1). This work was conducted in Lab A.

2.5. Dose-effect studies

Dose-effect experiments were carried out in two laboratories (Exp 2 in Lab A, and Exp 3 in Lab B). In Lab A, figure-eight mazes were used to obtain extensive dose-effect data using total motor activity as the dependent variable. Bifenthrin was administered using each of two dose volumes (1 or 5 ml/kg), two time points (4 or 7 h), and six to eight dose groups, including the vehicle control. The choice of four and 7 h dose-to-test time intervals was based on both the time-course data in Exp 1 and a desire to be consistent with previous work [32,55]. In Lab B, FOB testing was carried out using a similar experimental design, with the following exceptions: figure-eight maze testing was conducted after FOB assessments using similar apparatuses in Lab B.

2.6. Motor activity testing in the figure-eight maze (Exp 1–2–3)

Motor activity was monitored for 1 h using 16 (Lab A) or 8 (Lab B) figure-eight mazes, each consisting of a series of interconnected alleys (10 × 10 cm) converging on a central arena and covered with transparent acrylic plastic [35,41]. In Lab A, 5 min before being tested, the rats were placed into individual plastic cages with pine shavings and allowed to acclimate to the test room, which was maintained at the same environmental conditions as the animal colony and dosing room. In Lab B, there was no pre-testing acclimation period within the maze testing room. Horizontal and vertical activity were detected by phototransistor/photodiode pairs, eight equally spaced around the mazes at 0.5" above the floor (horizontal), and four pairs located 3.0" 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. Photobeam calibration was checked daily prior to testing.

2.7. Neurobehavioral evaluation using the FOB (Exp 3)

FOB testing began 3.5 or 6.5 h after dosing (taking approximately 4 min per rat), and rats were then placed in the motor activity chambers at 4 h and 7 h, the same time points as those tested in Exp 2. All FOB and subsequent motor activity tests in Lab B were conducted by the same observer, who had no knowledge of the treatment group for each animal. A modification of the FOB protocol [26] was used to best match the procedures used previously [32]. The rat was removed from the cage and evaluations were made on general appearance and salivation. The reactivity to handling was ranked. The rat was then placed on the top of a laboratory cart (open field) to explore for 2 min. The observer ranked the rat's arousal, gait score, tremors, head shaking, and pawing behavior. Both head shaking and pawing were ranked from one (none) to four (occurring most of the observation period). The pawing included forelimb slapping, grooming, and burrowing. Next, sensorimotor responses were ranked in response to two stimuli (click stimulus using a metal clicker and pinch of the tail using forceps). Forelimb grip strength and rectal temperature were then measured using appropriate devices. Finally, motor activity assessments were conducted.

2.8. Statistical analysis

Total activity was the dependent variable for all motor activity data. For Exp 1–2, continuous, motor activity data were analyzed by a general linear model (GLM; SAS, software release 6.12). Time-course data (Exp 1) were analyzed with a two-way ANOVA (treatment and testing time as independent variables) using data for which the testing times were similar for both dose volumes (i.e., 1, 3, and 7 h post-dosing). Mean contrasts were determined using Duncan's New Multiple Range Test. Dose-effect data (Exp 2 and 3) were analyzed with three-way ANOVAs (bifenthrin dose, dose volume, and testing time as independent variables) using the data for which the doses were similar for test times and dose volumes (i.e., Exp 2 — 1, 6, and 12 mg/kg; Exp 3 — (6, 12, and 20 mg/kg)).

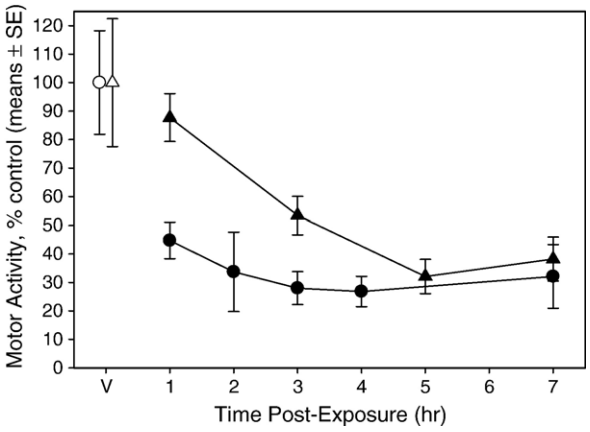

Fig. 1. The impact of dose volume on the time course of bifenthrin-induced suppression of motor activity. The effects of bifenthrin occurred faster and at a lower dose when administered at 1 ml/kg versus 5 ml/kg. Data are expressed as percent of respective time point controls. Open symbols represent the grand mean and SD for all the vehicle-only controls (V) for the 1 ml (circle) and 5 ml (triangle) groups. Filled symbols are bifenthrin exposed groups. [see text for details].

A severity score scheme was used to determine bifenthrin effects on the various functional outcomes examined using the FOB (Exp 3). The severity scoring method of analysis normalized individual data for all measures assigning scores from 1 (i.e., highly probable to occur in controls) to 4 (i.e., rarely observed in controls) [26,29]. The data from the FOB assessments were grouped in three behavioral domains of interest, as follows: 1) pyrethroid-related severity score, consisting of tremorigenic action, click response, pawing, head shaking and body temperature (signs of toxicity typically observed with pyrethroids), 2) neuromotor severity score, consisting of gait, grip strength, and motor activity assessments (indicating general motor performance), and 3) general reactivity score, including data from handling, tail pinch and arousal evaluations (generalized and non-specific depression). The composite scores were analyzed by three-way ANOVA (as described above) to test for main effects of dosage, testing time and dose volume as described for the motor activity counts.

Motor activity and FOB data were also analyzed using benchmark dose (BMD) quantification to calculate comparable potency estimates for the different experimental conditions [9,10]. BMD estimates were computed using benchmark dose software (BMDS, version 1.40c) [46]. A 30% decrease in motor activity was used as the benchmark response (BMR). This BMR was chosen to allow comparison of potencies with previous modeling of pyrethroid motor activity data [55]. Data sets were modeled using Hill (most cases), Polynomial-2, or Power models, whichever provided the best fit. A default p-value of 0.1 was used to test the homogeneity of group variances and the goodness of fit for modeling of means and variances. For FOB data, BMDs were calculated for pyrethroid-severity scores. The same system, procedures, and models applied to compute BMD values from motor activity data sets (see above) were used. In this case, an increase of 0.6 score units was used as the BMR. This BMR was considered an effect size comparable to the BMR30 used in modeling motor activity data.

3. Results

The bifenthrin doses used in this study resulted in decreased motor activity and clinical signs of toxicity. While significant changes were observed regardless of the dose volume or testing time, these two variables did influence potency. At the highest dosages a few rats showed transient periods of intense whole body tremors and hyper-reactivity. FOB results distinguished two periods of maximal effect depending on the dose volume. The 1 ml/kg groups were most affected 4 h post-dosing, whereas the 5 ml/kg groups were most affected 7 h post-dosing. All animals in the highest dose (20 mg/kg) in the 1 ml group exhibited fine tremors, periods of intense whole body tremor, and hyper-reactivity. The 26 mg/kg dose in the 5 ml group resulted in increased fine tremors and hyper-reactivity at the 7 h time point. There was a small increase in diarrhea in the higher volume animals compared to lower volume animals that was not related to dose, but was time dependent. Diarrhea was observed in 15 out 50 animals in the 5 ml 7 h group, and only one out of 50 animals in each of the other three groups. No rats died at any

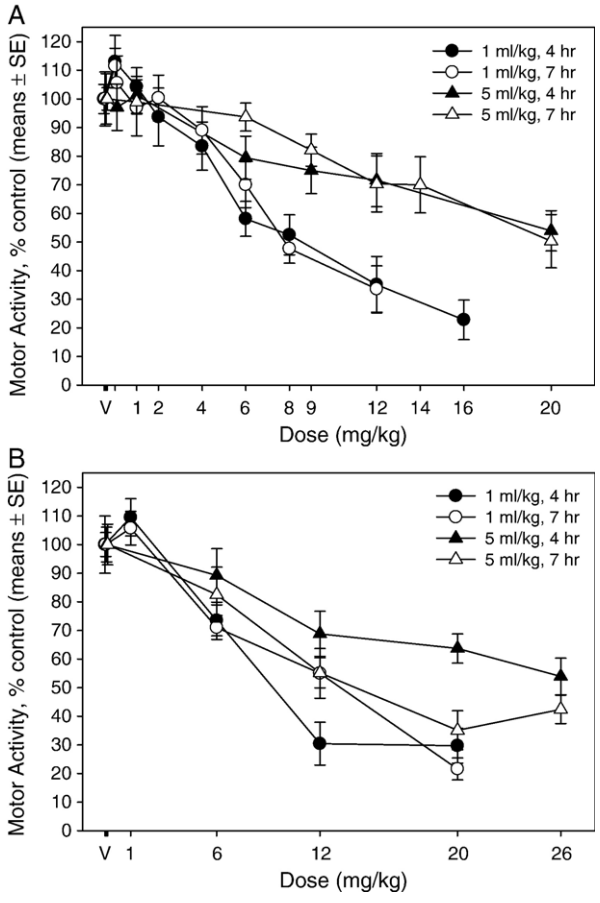

Fig. 2. The impact of dose volume and post-exposure test time on bifenthrin-induced suppression of motor activity. Dose-effect functions were characterized for 1 and 5 ml/kg dose volumes at 4 or 7 h post-exposure in two independent laboratories (Panel A — Lab A; Panel B — Lab B). Similar apparatuses were used in both laboratories. Bifenthrin was more potent at both post-exposure times when administered in the smaller dose volume. Data are expressed as percent of each group's respective vehicle-only (V) controls.

doses tested. All rats recovered to normal (by cage-side observations) within 24–48 h post-dosing.

Time-course studies revealed volume-dependent differences in the onset time of bifenthrin-induced decreases in motor activity (Fig. 1). The impact of dose volume is clearly evident with 1 ml/kg having a more rapid onset and a greater potency compared to the 5 ml/kg group, even though the dose in the 5 ml/kg group was 67% larger. At 1 ml/kg, a 50% decrease was observed as early as 1 h post-dosing, with a peak 74% decrease at 4 h post-dosing. Activity was still decreased (68%) at 7 h post-exposure. In contrast, at 5 ml/kg, the onset of effects took significantly longer, with only a 12% decrease at 1 h, and a peak-effect of 70% at 5–7 h. At 7 h there was an equivalent potency between the 12 and 20 mg/kg doses. These conclusions are supported by a significant interaction of treatment and testing time ($F(6,81)=3.15$, $p=0.008$). Mean contrast tests revealed significant differences between the 1 ml/kg and 5 ml/kg groups at 1 and 3 h ($p<0.05$), but not at 7 h ($p>0.05$).

Dose-effect experiments demonstrated dosage-related decreases in motor activity for all experimental conditions (Fig. 2A and B). Data from Lab A (Exp 2) demonstrates that potency is more influenced by dose volume than time post-exposure. Bifenthrin administered in 1 ml/kg was at least twice as potent at both 4 and 7 h compared to the 5 ml/kg groups (Fig. 2A). These conclusions are supported by a significant interaction of dose and volume ($F(3,176)=5.43$, $p=0.0050$). There were also significant main effects of dose ($F(1,176)=34.2$, $p=0.0001$) and volume ($F(1,176)=14.5$, $p=0.0002$). There was no significant interaction between dose and volume, nor was there a significant effect of test-time, or interaction of test-time with dose or volume (all p's>0.05). Results from motor activity testing in Lab B were mostly equivalent, with some exceptions (Fig. 2B). There was similar greater potency of 1 ml/kg compared to 5 ml/kg at both testing times. The 7 h potency difference was attenuated compared to the data from Lab A. Statistical analyses of these data revealed a significant interaction between dose volume and time of testing ($F(1,159)=9.14$, $p=0.003$), and significant main effects of dose ($F(3,159)=63.44$, $p=0.0001$) and volume ($F(1,159)=14.84$, $p=0.0002$). There was no significant interaction between dosage and volume, or dose and volume and time (all p's>0.05).

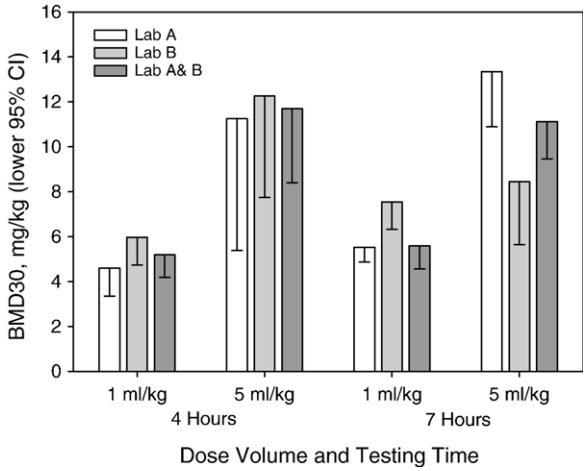

Fig. 3. BMD30 values for the effects of bifenthrin on motor activity. Benchmark dose values were computed for a 30% decrease in motor activity using the data from Fig. 2. BMD30 values, with the lower 95% confidence interval, are presented for data collected from Lab A (open bars), Lab B (light gray), and data from both labs combined (dark gray).

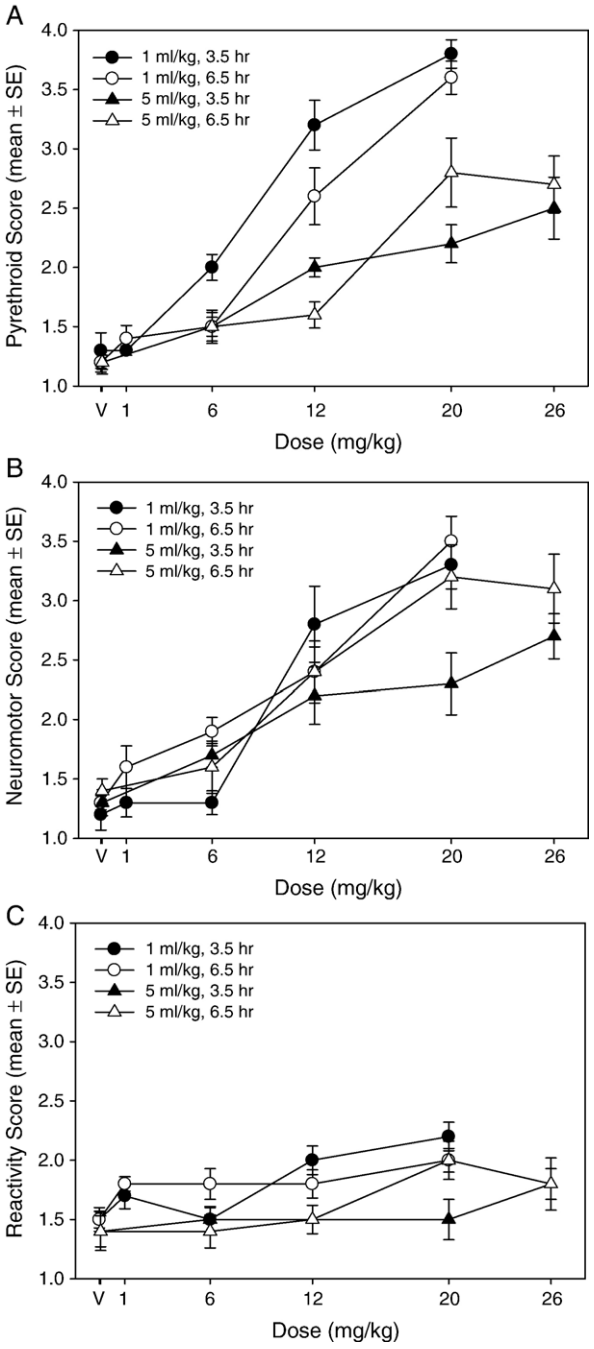

Fig. 4. The impact of dose and dose volume on the neurobehavioral effects of bifenthrin. Neurobehavioral function was evaluated using an FOB, with data grouped in three domains. A) Pyrethroid domain, B) Neuromotor domain, and C) Reactivity domain. Bifenthrin exposure increased the pyrethroid domain and neuromotor scores in a dose-related manner. [$n=10$ per group].

BMD analyses support the conclusion that increasing dose volume results in decreasing potency. Fig. 3 illustrates BMD30 values for Lab A and Lab B separately, and data from both labs

combined. At 4 h post-dosing, BMD30s were 2× greater in the 5 ml/kg groups for both labs. At 7 h post-dosing, BMD30s in the 5 ml/kg groups were almost 3× greater in Lab A, 20% higher in Lab B, and 2× greater when the data from the two labs was combined. These data demonstrate a 50% reduction in the potency of bifenthrin administered at a dose volume of 5 ml/kg compared to 1 ml/kg, regardless of dose-to-test times.

The influence of dose volume on the potency of bifenthrin on FOB endpoints was dependent on the behavioral domain (Fig. 4). There was a clear impact of dose and dose volume on the pyrethroid domain (Fig. 4A). There were dose-dependent increases in the pyrethroid domain score for all experimental groups. The lower dose volume was approximately 2-time more potent compared to the 5 ml/kg volume at both dose-to-test intervals. These conclusions are supported by significant main effects of dose ($F(3,159)=129.3$, $p=0.0001$), time ($F(1,159)=4.25$, $p=0.0411$), and volume ($F(1,159)=79.76$, $p=0.0001$), as well as significant interactions between dose and volume ($F(3,159)=16.5$, $p<0.0001$) and time ($F(1,159)=3.53$, $p=0.0165$). BMD estimates for a response change of 0.6 score units were consistent with the ANOVA results (Fig. 5). The effects of bifenthrin on the neuromotor and reactivity domains were dependent on both time and dose volume, with a greater impact of dose volume at 3.5 h compared to 6.5 h (Fig. 4). For the neuromotor domain there were overall significant main effects of dose ($F(3,159)=68.78$, $p=0.0001$) and volume ($F(1,159)=4.59$, $p=0.0338$), as well as an interaction between the two (i.e., dose-by-volume, ($F(3,159)=2.96$, $p=0.0344$). The effects of bifenthrin on the reactivity domain were minor, with small magnitude changes in severity (Fig. 4). There were main effects of dose ($F(3,159)=11.34$, $p=0.0001$) and volume ($F(1,159)=16.52$, $p=0.0001$), and a significant three-way interaction between dose, dosing volume and time ($F(3,159)=3.57$, $p=0.0157$).

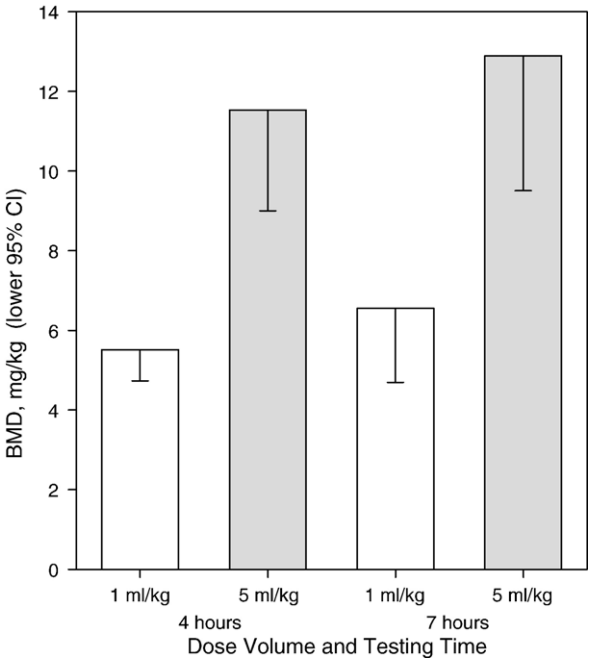

Fig. 5. BMD values for the effects of bifenthrin on the pyrethroid domain. Increasing dose volume resulted in a decrease in the potency of bifenthrin. Benchmark dose values were computed for a 0.6 unit increase in the domain score data presented in Fig. 4A. BMD values, with the lower 95% confidence interval, are presented for 1 ml (open bars), and 5 ml (gray bars) dose volumes and 4 and 7 h time points.

4. Discussion

Previous research has been inconsistent in estimating the acute neurotoxic potency of bifenthrin [32,55]. The current work tested the hypothesis that the inter-laboratory difference in potency is due to differences in dose volume. Comprehensive neurobehavioral assessments, including a wide array of neuromuscular, locomotor, and non-motor endpoints, were used to characterize the acute neurotoxicity of bifenthrin administered in two dose volumes, 1 ml/kg and 5 ml/kg. Results demonstrate that increasing dose volume decreases the potency and delays the time to onset of acute bifenthrin neurotoxicity. Data from automated motor activity assessments are consistent in demonstrating a 2-fold lower potency of bifenthrin when administered in the higher dose volume. Thus, volume differences may explain some of the inter-laboratory inconsistencies in potency estimates for the acute neurotoxicity of bifenthrin.

A previously published potency estimate for the neurobehavioral effects of bifenthrin was 3.2 mg/kg (1 ml/kg) based on a 30% reduction in motor activity [55]. This is consistent with the present findings of a BMD30 of 4.6 mg/kg (1 ml/kg) for motor activity and 5.5 mg/kg (1 ml/kg) for FOB. This estimate is much lower than a LOEL of 40 mg/kg (5 ml/kg) reported for the acute neurotoxicity of bifenthrin characterized with a FOB in an industry sponsored study [32].

There are a number of possible reasons for these differences in potency. Dose volume was influential in determining potency for both motor activity and FOB, with the higher dose volume being about 50% less potent. The reasons for the volume dependent toxicity of bifenthrin are not currently known. The differences are not due to chemical composition of the tested compounds. The bifenthrin used in both studies was of similar purity and isomeric ratios (FMC Corp, personal communication). The vehicle used was not responsible, since the vehicle in both cases was corn oil. Depending on the chemical, increasing dose volume can decrease [25] or increase [12] toxicity. The relationship between dose volume and toxic potency of bifenthrin is likely due to a complex interaction of gastrointestinal factors that influence absorption of fat soluble chemicals, including: the impact of concentration on absorption rates [33], volume or solvent induced diarrhea which increases gut motility [25], direct toxicity of the chemical to intestinal mucosal cells [51], the role of transport mediated uptake [27], and possible metabolism by enteric esterases and cytochrome P450s [36,37]. Volume induced diarrhea may be partially responsible for the decreased potency, in that an increased incidence of diarrhea was present in the high-dose volume group at the 7 h time point, but not at the 4-h time point. A toxicokinetics study of the impact of vehicle volume on bifenthrin absorption and distribution would answer a number of these questions.

The inter-laboratory differences in bifenthrin potency are not entirely explained by the current findings. There is a 12-fold

potency difference in previous reports [32,43,55]. The current data demonstrate that dose-volume differences result in only a 2-fold difference in potency. Therefore, there must be additional differences between studies that drive potency differences. There are a variety of organismic and experimental factors known to cause differences in toxicity between laboratories. These variables include, but are not limited to, strain of rat, diet, social setting, environmental variables (e.g., temperature, humidity, lighting, noise, housing), and staff training [4,5,18,19,42,44]. The limited published data on the acute neurobehavioral effects of bifenthrin make it difficult to speculate on which of these variables may be responsible for the inter-laboratory differences in the potency.

In summary, the current work tested the hypothesis that inter-laboratory differences in the potency of bifenthrin are due to differences in dose volume. Results demonstrate that this potency difference is partially due to differences in dose volume. Increasing dose volume delays the time to onset and decreases the potency of acute bifenthrin neurotoxicity. This finding demonstrates that vehicle volume is an important variable that must be considered when comparing data from different studies. These data also suggest a need for standardization of both vehicle and volume in toxicity studies.

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