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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