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Environmentally relevant pyrethroid mixtures: A study on the correlation of blood and brain concentrations of a mixture of pyrethroid insecticides to motor activity in the rat

Michael F. Hughes[a,*], David G. Ross[a], James M. Starr[b], Edward J. Scollon[a,1], Marcelo J. Wolansky[c,2], Kevin M. Crofton[a,3], Michael J. DeVito[a,4]

[a] U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC, United States
[b] U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Research Triangle Park, NC, United States
[c] National Research Council, Research Triangle Park, NC, United States

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ABSTRACT

Human exposure to multiple pyrethroid insecticides may occur because of their broad use on crops and for residential pest control. To address the potential health risk from co-exposure to pyrethroids, it is important to understand their disposition and toxicity in target organs such as the brain, and surrogates such as the blood when administered as a mixture. The objective of this study was to assess the correlation between blood and brain concentrations of pyrethroids and neurobehavioral effects in the rat following an acute oral administration of the pyrethroids as a mixture. Male Long-Evans rats were administered a mixture of β-cyfluthrin, cypermethrin, deltamethrin, esfenvalerate and *cis*- and *trans*-permethrin in corn oil at seven dose levels. The pyrethroid with the highest percentage in the dosing solution was *trans*-permethrin (31% of total mixture dose) while deltamethrin and esfenvalerate had the lowest percentage (3%). Motor activity of the rats was then monitored for 1 h. At 3.5 h post-dosing, the animals were euthanized and blood and brain were collected. These tissues were extracted and analyzed for parent pyrethroid using HPLC-tandem mass spectrometry. Cypermethrin and *cis*-permethrin were the predominate pyrethroids detected in blood and brain, respectively, at all dosage levels. The relationship of total pyrethroid concentration between blood and brain was linear ($r = 0.93$). The pyrethroids with the lowest fraction in blood were *trans*-permethrin and β-cyfluthrin and in brain were deltamethrin and esfenvalerate. The relationship between motor activity of the treated rats and summed pyrethroid blood and brain concentration was described using a sigmoidal E_{max} model with the Effective $Concentration_{50}$ being more sensitive for brain than blood. The data suggests summed pyrethroid rat blood concentration could be used as a surrogate for brain concentration as an aid to study the neurotoxic effects of pyrethroids administered as a mixture under the conditions used in this study.

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1. Introduction

Pyrethroids are a class of synthetic insecticides with a structure based on the botanical pyrethrins. The structural commonality of pyrethrins and pyrethroids are the acid and alcohol moieties that are linked by an ester group. Another common feature of pyrethrins and pyrethroids is that they may have 1–3 chiral carbons and are isomeric (Soderlund et al., 2002). Consequently, there may be differences in the metabolism of the isomers (hydrolysis vs. oxidation) as well as insecticidal potency (Miyamoto, 1990; Soderlund et al., 2002). In general, pyrethroids tend to have greater insecticidal activity and are less susceptible to

* Corresponding author at: U.S. Environmental Protection Agency, MD B105-03, Research Triangle Park, NC 27709. United States.
E-mail address: hughes.michaelf@epa.gov (M.F. Hughes).
[1] Current address: Syngenta Crop Protection, Greensboro, NC, United States.
[2] Current address: University of Buenos Aires and Argentine NRC Institute IQUIBICEN, Buenos Aires, Argentina.
[3] Current address: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, NC, United States.
[4] Current address: National Institute of Environmental Health Sciences, National Toxicology Program, Research Triangle Park, NC, United States.

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environmental degradation than pyrethrins (Bradberry et al., 2005).

The many uses of pyrethroids including agricultural, commercial and residential pest control, and veterinary and medical practices (Amweg et al., 2005; Bradberry et al., 2005) may lead to human exposure. Residues of multiple pyrethroids are detected in surface wipe samples collected from child care centers (Tulve et al., 2006) and residential homes (Stout et al., 2009), indoor air and dust samples (Rudel et al., 2003), and on fruits and vegetables (USDA, 2014). A biological monitoring study in Canada of a metropolitan populace exposed to pyrethroids in the diet found that the study population was mainly exposed to permethrin and cypermethrin (Fortin et al., 2008). From their use in agriculture and pest management, humans can be exposed to multiple pyrethroids (Fortin et al., 2008; Heudorf and Angerer, 2001; Stout et al., 2009; Tulve et al., 2006; Tornero-Velez et al., 2012).

Most pyrethroids are commonly classified into two groups, termed Type I and II, based on chemical structure and neurotoxic effects in rodents (Soderlund et al., 2002; Soderlund, 2012). Type I pyrethroids (e.g., permethrin, bifenthrin) contain either a primary or secondary alcohol, and their neurotoxic syndrome hallmark is tremor. Type II pyrethroids (e.g., cypermethrin and deltamethrin) are primary alcohols with a cyano group on the alpha-carbon of the alcohol. Oral administration of exposure to Type II pyrethroids results in choreoathetosis and salivation.

The mode of action of Type I and II pyrethroids appears to be binding to and disruption of voltage-gated sodium channels in targeted neurons (Soderlund et al., 2002; Soderlund, 2012). Wolansky et al. (2006) conducted extensive dose-response assays using motor activity as a neurobehavioral endpoint in rats exposed to eleven pyrethroids administered by oral gavage individually; all compounds produced a dose-related decrease in motor activity, but the potency was variable, dependent upon the pyrethroid administered. In a later study, Wolansky et al. (2009) reported dose-additive effects in rats exposed to an eleven chemical mixture of Type I and II pyrethroids for a decrease in motor activity. An in vitro study by Cao et al. (2011) reported dose-additive effects in inducing sodium influx in primary cultures of murine cerebrocortical neurons with this same pyrethroid mixture. However, based on several other *in vivo* and *in vitro* studies, Breckenridge et al. (2009) proposed that Type I and Type II compounds have separate mechanisms of neurotoxicity. Nevertheless, the U.S. Environmental Protection Agency in 2011 determined the pyrethroids share a common mechanism of action for a cumulative risk assessment (US EPA, 2011).

The nervous system is the primary target tissue for the neurotoxicity produced soon after acute exposure to pyrethroids in laboratory animals. Mice and rats administered very low doses of pyrethroids by direct infusion into the brain display pyrethroid poisoning signs, including tremors (Lawrence and Casida, 1982; Gray and Rickard, 1982a). Neurotoxicological endpoints such as tremors and decreased motor activity in rats administered deltamethrin (i.v.) (Gray and Rickard, 1982b) and bifenthrin (p.o.) (Scollon et al., 2011), respectively, are correlated with brain concentrations of these pyrethroids. As humans are exposed to multiple pyrethroids (Fortin et al., 2008; Heudorf and Angerer, 2001; Stout et al., 2009; Tulve et al., 2006; Tornero-Velez et al., 2012), it is important to understand the disposition of these pesticides, particularly to target organs such as the brain. In the present work we examined the distribution to blood and brain of an environmentally-relevant mixture of five pyrethroid compounds after an acute oral gavage in adult rats (Table 1). The objectives of this study were to: (1) determine the blood and brain concentrations of the test chemicals to characterize the relationships between administered mixture dose and target tissue level; (2) assess the correlation of blood and brain concentrations of the administered pyrethroids to motor activity, a behavioral end point. Only the concentrations of parent pyrethroids were determined as metabolism is thought to be a principal detoxication mechanism in pyrethroid intoxications in mammals (Soderlund et al., 2002). Information on dose to target tissue of a mixture of pyrethroids and relating it to a behavioral effect may reduce uncertainties in the cumulative health risk assessment of this class of insecticides.

2. Materials and methods

2.1. Chemicals

The selection process for the five pyrethroids used in this study has been described by Tornero-Velez et al. (2012). The choice of pyrethroids used in this study was based on a national study of a randomly selected set of 168 child-care centers from across the United States (Tulve et al., 2006). Basically, the five pyrethroids had a greater frequency of occurrence and made up roughly 95% of the pyrethroid load found in the Tulve et al. (2006) study.

Each pyrethroid used in the dosing solution was provided by its respective manufacturer as follows: permethrin and cypermethrin (FMC Corporation, Philadelphia, PA); deltamethrin and β-cyfluthrin (Bayer CropScience, Research Triangle Park, NC); and esfenvalerate (DuPont Crop Protection, Wilmington, DE). The physical and chemical properties of the pyrethroids used in this study have been described previously (Wolansky et al., 2006).

The solvents used for processing samples included acetone, hexanes, ethyl acetate, methanol (Fisher Scientific, Pittsburgh, PA), cyclopentane and acetonitrile (Honeywell Burdick & Jackson, Muskegon, MI). These solvents were pesticide grade or better. The water used for all sample analysis had a resistance of 18 MΩ. Corn oil was purchased from Sigma (St. Louis, MO; Product Number C8267).

Primary calibration standards including *cis*-permethrin (99% purity), *trans*-permethrin (94%), deltamethrin (99%), cypermethrin (98%), β-cyfluthrin (98%) and esfenvalerate (98%) were purchased from Absolute Standards (Hamden, CT). Ring-labeled (phenoxy-$^{13}C_6$) pyrethroids, used as internal standards or surrogates, were purchased from Cambridge Isotope Laboratories (Andover, MA). These labeled standards included *cis*-permethrin, *trans*-permethrin, β-cyfluthrin and cypermethrin.

2.2. Animals

Male Long-Evans rats (Charles River Laboratories, Wilmington, MA) were obtained at 55–58 days of age, and housed two per cage

Table 1
Pyrethroid type, percentage of dose in mixture and potency of pyrethroids administered as a mixture to rats.

	β-Cyfluthrin	Cypermethrin	Deltamethrin	Esfenvalerate	Permethrin[b]
Type	II	II	II	I	I
% of total mixture dose	12.9	28.8	3.4	2.7	52.2
Relative potency[a]	1.136	0.235	1.000	2.092	0.059

[a] Relative potency based on a pyrethroid ED_{30} for effect on motor activity relative to deltamethrin as the index pyrethroid (Wolansky et al., 2006).
[b] Relative potency for permethrin determined from 40:60 *cis:trans*-permethrin.