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Relationship between exposure, body burden and target tissue concentration after oral administration of a low-dose mixture of pyrethroid insecticides in young adult rats

Mónica Elizabeth Mosquera Ortega[a,c], Delfina Mercedes Romero[a,c,1], Alejandro Martín Pato[a], Carla Solange Sosa-Holt[a], Adriana Ridolfi[b], Edda Villaamil Lepori[b], Marcelo Javier Wolansky[a,c,*]

[a] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Argentina
[b] Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina
[c] IQUIBICEN Institute, Consejo Nacional de Investigaciones Científicas, CONICET, Argentina

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ABSTRACT

Pyrethroids (PYRs) are synthetic insecticides increasingly used in agricultural and household pest control. Little is known on how the toxicity of highly effective bolus doses of single compounds compares to more realistic scenarios of low-level exposure to PYR mixtures. In this study, we examined a quaternary mixture of two noncyano (tefluthrin, TEF; bifenthrin, BIF) and two cyano (α-cypermethrin, α-CPM; deltamethrin, DTM) PYRs in young adult rats. These compounds are mostly composed of PYR isomers ranking top ten in acute lethality in rats. Concurrently, we administered near-threshold levels of the four PYRs dissolved in corn oil by oral route. Six hours later blood was collected and the liver and cerebellum were dissected out to determine PYR concentrations in these tissues using Gas Chromatography with Electron Capture Detector (GC-ECD). The mixture caused mild-to-moderate changes in non-locomotor behaviors and subcutaneous body temperature (up to +1.2–1.5 °C increase at 2–4 h after dosing, respectively, compared to pre-dosing records). The most toxic PYRs BIF and TEF reached higher concentrations in the cerebellum than the cyano-compounds α-CPM and DTM. In addition, PYR concentrations in the cerebellum were correlated to single compound proportions in the dosing solution and changes in body temperature. Our results suggest that aggregate exposures resulting in a target tissue burden of $\sim 10^{-1}$ nmoles PYR/g may be toxicologically relevant, expanding the evidence on exposure-dose-effect relationships for PYRs, and serving to design convenient pharmacokinetic models for environmentally relevant exposures to PYR mixtures.

1. Introduction

Pyrethroids (PYRs) are synthetic structural derivatives of a series of natural compounds with insecticidal activity named pyrethrins (Casida, 1980; Elliott, 1976). Most PYRs have been classified as Type I and Type II according to their chemical structure and acute neurotoxic effects in small rodents. Type I compounds lack an α-cyano group on the phenoxybenzyl moiety, and cause intense tremors (T-syndrome) in rats. Type II compounds contain an α-cyano group on the alcohol moiety, and cause repetitive bursts of pawing and burrowing, crawling, choreoathetosis, and profuse salivation as the dose administered increases (CS-syndrome). There are a few PYRs that produce mixed signs, including tremors and salivation, and have been accordingly classified as Type I/II (Soderlund et al., 2002; Wolansky and Harrill, 2008). Type I, type II and mixed-type PYRs have been long proposed to share a common primary mode of neurotoxic action. PYRs prolong inward sodium currents at voltage-gated sodium channels (VGSC) in targeted neurons. Thus, in conscious animals acute exposure to PYRs may induce prolonged nervous system hyperexcitation leading to neurophysiological collapse (Narahashi, 2000; Soderlund, 2012). Extrapolation from high dose toxicokinetics and effects of single PYRs in experimental animals to more realistic low-level exposure scenarios in humans requires the consideration of several influential factors. The canonical type I/II classification is mostly based on studies using single high bolus

* Corresponding author at: Laboratorio QB48, Pabellón 2, Piso 4, Campus Ciudad Universitaria UBA, Ciudad Autónoma de Buenos Aires, C1428EGA, Argentina.
E-mail addresses: monicaemosquera@gmail.com (M.E. Mosquera Ortega), delfina.romero@inserm.fr (D.M. Romero), alejandropato@gmail.com (A.M. Pato), carlasosaholt@gmail.com (C.S. Sosa-Holt), asridolfi@yahoo.com.ar (A. Ridolfi), evillaam@ffyb.uba.ar (E. Villaamil Lepori), mjwolansky@qb.fcen.uba.ar (M.J. Wolansky).
[1] Current address: Cortical Development and Pathology, Institut du Fer a Moulin, INSERM UMRS 839, 17 Rue du Fer a Moulin, 75005 Paris, France.

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doses administered by intravascular (iv) or intracerebral (ic) routes in mice and rats, with clinical syndromes fully evolving through < 1 h after dosing (Lawrence and Casida, 1982; Verschoyle and Aldridge, 1980). A more diverse repertoire of neurobehavioral signs may appear along a few hours after oral exposure to PYRs in young adult rats (Wolansky and Harrill, 2008). Mild exacerbation of both motor activity and stereotyped behavior is observed soon after oral administration of middle-to-high effective doses, followed by dose-dependent decreases in activity later, regardless of the compound structure (Crofton and Reiter, 1988, 1984; Wolansky and Harrill, 2008). Moreover, low-effective exposure to PYRs causes mild increase in the core body temperature during the initial 30–90 min after oral dosing, regardless of the type, although compound-specific dose-related alterations in this endpoint (intense hyper- and hypothermia caused by Type I and Type II PYRs in adult rats, respectively) are observed after high-effective exposure at 120–180 min (McDaniel and Moser, 1993; Soderlund et al., 2002; Wolansky and Harrill, 2008; Wolansky et al., in preparation). PYRs may certainly have different actions and threshold levels in rats depending on the exposure conditions and the neurobehavioral endpoint (Wolansky and Harrill, 2008; Wolansky and Tornero-Velez, 2013). In humans, PYRs enter the body mostly via the oral route (pesticide residues in food; hand-to-mouth behavior in young children), and through the inhalation of environmental residues after the household pest control application of products containing PYRs as active ingredients (ATSDR, 2003; Julien et al., 2008; Li et al., 2014; Morgan, 2012; Tulve et al., 2006). Moreover, it is worth mentioning that environmental and human studies indicate that different patterns of combined exposure to PYRs may occur in general population (Haines et al., 2017; Morgan, 2012; Soderlund, 2012; Tornero-Velez et al., 2012b). A comprehensive understanding of health risks through the exposure to relevant mixtures of PYRs may thus require the assessment of different dosing and testing conditions (Wolansky and Tornero-Velez, 2013).

There are some gaps in the information about the existing relationship between PYR sample composition, absorption and distribution to target tissues, and dosage-related variations in the observed toxicity in rats and mice. In adult rats, brain concentration at ~6-9 h after oral exposure to the noncyano PYR bifenthrin (BIF) directly correlate with the severity of BIF actions in motor activity observed a few hours earlier (Scollon et al., 2011; Wolansky et al., 2007b). The same laboratory further examined the relationship between the dose administered, the tissue level (i.e., blood, liver, fat, and brain) and the motor activity alteration after acute oral joint exposure to low-effective doses of five PYRs (Hughes et al., 2016a; Starr et al., 2014, 2012). The test mixture in these studies consisted of a mix of isomer-rich compounds (deltamethrin [DTM] and esfenvalerate, both mostly consisting of 1 isomer; and β-cyfluthrin, featuring 2 out of 8 possible isomers), and racemic samples (cypermethrin [CPM] and permethrin, consisting of eight and four isomers, respectively). The brain was the tissue where individual PYR concentrations correlated best with single-compound ratios in the mixture dosing solution. Various toxicokinetic (TK) factors such as absorption rates, intestinal metabolism and decomposition mechanisms, and hepatic and blood binding proteins were proposed to contribute to PYR structure- and isomer-specific tissue disposition findings. Hence, a question worth asking is to what extent the mixture composition of the dosing solution and the testing endpoint may influence the relationship between PYR disposition into tissues and neurotoxicity. In this work, we evaluated a low-dose mixture of two CS-syndrome and two T-syndrome compounds in young adult rats to characterize the relationship between the administered dose, the target tissue dose and the effects of PYRs using subcutaneous body temperature as an endpoint.

2. Materials and methods

2.1. Animals

Hsd:WI Wistar rats (Animal Colony, Universidad de Buenos Aires; FCEN-UBA) were obtained at 8–9 weeks of age. As soon as they were received, all animals were housed two per cage in polycarbonate cages (45 cm × 24 cm × 20 cm) containing heat sterilized pine shavings, controlling for body weight balance between cages. All animals were maintained in the colony rooms on a 12:12 h photoperiod (0600:1800) at 22.5 ± 2.5 °C. Feed and tap water were provided ad libitum except when indicated. Experimental protocols were approved by UBA School of Science, Hygiene and Safety Department. Procedures recommended by NRC's Guide for the Care and Use of Laboratory Animals (8th edition) and FCEN-UBA Animal Colony Direction were followed to ensure reducing animal suffering to the least possible.

2.2. Chemicals

Test chemical samples were analytical grade (≥99% purity) except TEF (96.3% purity). BIF (CASRN 82657-04-3), 2-methyl-1,1-biphenyl-3-yl-methyl-(Z)-(1R)-cis-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropane-carboxylate, consisted of 99% +(Z)-(1R)-cis isomer. DTM (CASRN 2918-63-5), (*S*)-α-cyano-3-phenoxybenzyl-(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane-carboxylate, consisted of 98% +(*S*)-(1R)-cis isomer). α-CPM (CASRN 67375-30-8), (RS)-α-cyano-3-phenoxybenzyl-(1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate, is reported to be made up of +90% 1R and 1S configuration of the most active enantiomeric pair of the cis isomers of CPM (Pronk et al., 1996). BIF, DTM and α-CPM were purchased from ChemService (West Chester, PA, USA). TEF (CASRN 79538-32-2), 2,3,5,6-tetrafluoro-4-methylbenzyl-(Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2- dimethylcyclopropanecarboxylate, comprising equal amounts of the enantiomeric pair of +(Z)-(1R)-cis isomers (Knaak et al., 2012), was generously provided by Syngenta Argentina. The chemical structure and isomer composition of these PYRs are presented in Fig. 1. Chlorpyrifos (CASRN 2921-88-2), *O*,*O*-diethyl-O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate, used as an internal standard in the gas chromatographic determination of PYRs, was purchased from ChemService (West Chester, PA, USA). All organic solvents were of pesticide grade quality (Aberkon Química, Buenos Aires, Argentina).

2.3. Test mixture

The test mixture was intended to replicate a worst-case example of concurring exposure to pest control products formulated with highly toxic isomers of modern PYRs. Four criteria were used to select the number and identity of the compounds mixed up to prepare the test mixture. First, we selected four of the most toxic PYRs based on oral LD50 in adult rats (Wolansky and Harrill, 2008; WHO, 2010). Second, we considered the results of the First National Environmental Health Survey of Child Care Centers (CCC Survey; Tulve et al., 2006). This survey designated 334 child care buildings, from which 168 completed the survey. Tornero-Velez et al. (2012a,b) used a rigorous mathematical modeling and statistical analysis to characterize the distribution of PYR residues in the CCC study. These authors found two, three and four PYR compounds simultaneously occurring at 30, 15 and 10% of the CCC sites, respectively; co-occurrence of ≥5 PYRs at detectable levels was ≤ 2.5% of the total CCC sites sampled. Third, the detection frequency and maximum residue loading of DTM and CPM ranked top-ten among the PYRs analyzed in several environmental studies and food residue surveys (Jardim and Caldas, 2012; Morgan, 2012; Tulve et al., 2011, 2006). Last, we combined cyano and noncyano PYRs to blend the most common type-specific neurobehavioral syndromes that these insecticides may cause in rats (Wolansky and Harrill, 2008). Accordingly,