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PBPK modeling of the *cis*- and *trans*-permethrin isomers and their major urinary metabolites in rats

Marie-Emilie Willemin [a,b,1], Sophie Desmots [c], Rozenn Le Grand [d], François Lestremau [e], Florence A. Zeman [a], Eric Leclerc [b], Christian Moesch [d], Céline Brochot [a,*]

[a] Institut National de l'Environnement Industriel et des Risques (INERIS), Unité Modèles pour l'Ecotoxicologie et la Toxicologie (METO), Parc ALATA BP2, 60550, Verneuil en Halatte, France
[b] Sorbonne University, Université de Technologie de Compiègne, CNRS, UMR 7338 Biomechanics and Bioengineering, Centre de recherche Royallieu CS 60319,60203 Compiègnee Cedex, France
[c] Institut National de l'Environnement Industriel et des Risques (INERIS), Unité Toxicologie Expérimentale (TOXI), Parc ALATA BP2, 60550, Verneuil en Halatte, France
[d] Centre Hospitalo-Universitaire de Limoges, Service de Pharmacologie et de Toxicologie — Pharmacovigilance, 2, avenue Martin Luther King, 87042 Limoges, France
[e] Institut National de l'Environnement Industriel et des Risques (INERIS), Unité Innovation pour la Mesure (NOVA), Parc ALATA BP2, 60550, Verneuil en Halatte, France

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ABSTRACT

Permethrin, a pyrethroid insecticide, is suspected to induce neuronal and hormonal disturbances in humans. The widespread exposure of the populations has been confirmed by the detection of the urinary metabolites of permethrin in biomonitoring studies. Permethrin is a chiral molecule presenting two forms, the *cis* and the *trans* isomers. Because *in vitro* studies indicated a metabolic interaction between the *trans* and *cis* isomers of permethrin, we adapted and calibrated a PBPK model for *trans*- and *cis*-permethrin separately in rats. The model also describes the toxicokinetics of three urinary metabolites, *cis*- and *trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis*- and *trans*-DCCA), 3-phenoxybenzoic acid (3-PBA) and 4′OH-phenoxybenzoic acid (4′-OH-PBA). *In vivo* experiments performed in Sprague–Dawley rats were used to calibrate the PBPK model in a Bayesian framework. The model captured well the toxicokinetics of permethrin isomers and their metabolites including the rapid absorption, the accumulation in fat, the extensive metabolism of the parent compounds, and the rapid elimination of metabolites in urine. Average hepatic clearances in rats were estimated to be 2.4 and 5.7 L/h/kg for *cis*- and *trans*-permethrin, respectively. High concentrations of the metabolite 4′-OH-PBA were measured in urine compared to *cis*- and *trans*-DCCA and 3-PBA. The confidence in the extended PBPK model was then confirmed by good predictions of published experimental data obtained using the isomers mixture. The extended PBPK model could be extrapolated to humans to predict the internal dose of exposure to permethrin from biomonitoring data in urine.

1. Introduction

Pyrethroid insecticides are used in household applications, agriculture or medicine (US EPA, 2011). Their consumption increased recently, probably in response to restrictions on the use of other insecticides such as organophosphates and organochlorines. Because pyrethroids are rapidly metabolized, human exposure is usually assessed by measuring the urinary concentrations of their metabolites, including 3-phenoxybenzoic acid (3-PBA) and *cis*- and *trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis* and *trans*-DCCA). These metabolites can either be specific to a pyrethroid (e.g., 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid for deltamethrin) or common to several ones (e.g., 3-PBA). The widespread exposure of humans was confirmed in biomonitoring studies, carried out in different countries such as France, Germany, USA and Japan, where the biomarkers of exposure were detected in almost all the individuals tested (Egerer et al., 2004; Heudorf et al., 2006; Barr et al., 2010; Ueyama et al., 2010; Morgan, 2012; InVS, 2013). In particular, these different studies demonstrated the large exposure of the French population to pyrethroids since the common metabolite 3-PBA was recovered at high amounts in urine with a 2 and 3-factor compared to the other countries.

Among pyrethroids, permethrin (3-phenoxybenzyl (*1RS*,*3RS*;*1RS*,*3SR*)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) is one of the most commonly used especially in household applications (US EPA, 2005; Stout et al., 2009). Like other pyrethroids, permethrin acts on the nervous system of insects and mammals by interfering with neuronal voltage-gated sodium channels to disrupt the function of neurons (Soderlund et al., 2002; Soderlund, 2012). In mammals, permethrin is suspected to induce disturbances on the neuronal and hormonal systems. Syndrome T (aggressive sparring, fine tremors) (Verschoyle and Aldridge, 1980; Wolansky and

* Corresponding author.
E-mail address: celine.brochot@ineris.fr (C. Brochot).
[1] Present address: US Food and Drug Administration (FDA), National Center for Toxicological Research (NCTR), Division of Biochemical Toxicology, 3900 NCTR Road, Jefferson, AR 72079, United States.

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Harrill, 2008) and reproductive modifications, especially on testosterone levels (Zhang et al., 2007; Zhang et al., 2008; Jin et al., 2012) were observed in rats after acute and chronic exposures respectively. In humans, the reported adverse effects are skin irritation, paraesthesia or headaches depending on the routes of exposure (Lequesne et al., 1981; Flannigan et al., 1985; He et al., 1989; Gotoh et al., 1998; Bradberry et al., 2005). Epidemiological studies have also shown associations between modifications of the semen quality and the presence of pyrethroids metabolites in urines (Meeker et al., 2008; Young et al., 2013; Imai et al., 2014).

Permethrin has four stereoisomers due to the two chiral carbons on the cyclopropane rings. The *cis*- and *trans*-isomers are comprised of enantiomers 1R,S-*cis*-permethrin and 1R,S-*trans*-permethrin. Metabolic interactions between the *cis* and *trans* isomers of permethrin have been previously observed *in vitro* using hepatic microsomes (Scollon et al., 2009). The intrinsic hepatic clearances of the binary mixture were found to be reduced compared to that of the individual isomers; by 39% for *trans*-permethrin in rats and a factor 2 for both isomers in humans. Such metabolic interactions could contribute to the increased time of residence of the permethrin isomers (active form) in the body potentially predisposing the organism to its associated risks. Since the ratio of the permethrin isomers in the environment is not precisely known and is not constant, it is necessary to characterize first the toxicokinetics of each isomer separately and then link them using a specific model that accounts for their metabolic interactions.

In this paper, we propose to develop and calibrate a PBPK model for *cis*- and *trans*-permethrin in a Bayesian framework using data obtained from new *in vivo* experiments in rats exposed to the individual isomers. The PBPK model developed here is an extension of the PBPK models previously published (Tornero-Velez et al., 2012; Wei et al., 2013). The extended PBPK model includes the kinetic of the isomer of permethrin in additional organs and integrates the toxicokinetics of the three urinary metabolites that are commonly used as biomarkers of permethrin exposure (3-PBA, *cis*- and *trans*-DCCA) and the metabolite 4′OH-phenoxybenzoic acid (4′-OH-PBA) a major metabolite of permethrin (Takaku et al., 2011).

2. Materials and methods

2.1. Chemicals

Cis-permethrin (3-phenoxybenzyl(1RS)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, 99.4% purity) and *trans*-permethrin (3-phenoxybenzyl (1RS)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 99% purity) were obtained from ChemService (West Chester, USA) and Dr. Ehrenstorfer (Augsburg, Germany), respectively. The internal standards *cis*-[$^{13}C_6$]permethrin (98% purity) and *trans*-[$^{13}C_2$]DCCA (98% purity) were purchased from LGC standard (Cambridge Isotope Laboratories, USA). Corn oil was acquired from Sigma-Aldrich (St Quentin Fallavier, France).

2.2. Animals

Adult male Sprague–Dawley rats were purchased from Janvier (Genet de lisle, France). They weighed 468 g ± 21 g (mean body weight (BW) ± standard deviation (SD)) and were 90–100 days old at the time of the experiments. The experimental protocol was approved by an internal ethics committee. Each rat was housed in a cage with a 12 h light/12 h dark cycle at ambient temperature (22 °C ± 2 °C) and relative humidity (55 ± 15%). Food (Altromin for rat and mouse, Genestril, Royaucourt, France) and tap water were provided *ad libitum*. The rats were allowed a minimum acclimation period of 4 days before experiments. Rats were not fasted before the administration of permethrin, as it was previously done for toxicokinetic studies on deltamethrin (Godin et al., 2010) and permethrin (Tornero-Velez et al., 2012).

2.3. Experiments

A dose of 25 mg/kg of either *cis*- or *trans*-permethrin dissolved in corn oil (2 mL/kg) was administered to rats by gavage. The same solvent (corn oil) as the one used in previous toxicokinetic studies on pyrethroids was kept to insure reliable comparisons, since the nature of the solvent can have an impact on the absorption process in case of highly lipophilic chemicals like permethrin (Soderlund et al., 2002). Based on our preliminary study (Lestremau et al., 2014), at the dose of 20 mg/kg of permethrin, both parents and metabolites were quantified in most of the matrices with our analytical procedure, and no side effects were observed in rats. The dose was increased in our current study by a 0.25-fold to insure the quantification of all the compounds in all the matrices. Access to food was provided 3 h after dosing. Groups of 4 rats were sacrificed with CO_2 at 0.5, 1, 1.5, 2, 4, 6, 10, 24, 48 h and 6 days after administration. For the time points below 24 h, the rats were housed individually in a metabolic cage until euthanasia with CO_2. Because the time residence of the rats in the metabolic cage was limited to 24 h consecutively, several groups of rats were considered for the time points 48 h and 6 days. Urine and feces were then collected on 24 h intervals and were then cumulated.

Immediately after the euthanasia of the rat, blood samples were drawn from the inferior vena cava and collected in heparinized tubes. Formic acid 1% was added *v*/*v* to blood to inhibit the metabolism of permethrin due to carboxylesterases (CE) enzymes and to preserve the stability of the compounds. The median liver lobe, the perirenal fat, the muscle of the right thigh, the right kidney, the right testis and the brain were isolated and weighed. Urine and feces were collected every day in a metabolic cage until the 6th day. All matrices were frozen at −80 °C until analysis. No precautions were taken during the collection of the urine since no specific procedures were described for similar studies on pyrethroids metabolites in the literature (Kühn et al., 1996; Leng and Gries, 2005).

2.4. Chemical analyses

The extraction and detection of analytes in organs and feces were performed by GC–MS/MS based on the analytical method developed by Lestremau et al. (2014). Parent compounds *cis*- and *trans*-permethrin were dosed in each matrix and the metabolites *cis*- and *trans*-DCCA in blood, liver and feces. All the organs were thawed and ground with a mortar and a pestle and then with microbeads using a Precellys homogenizer (Bertin, Montigny le Bretonneux, France). The internal standards *cis*-[$^{13}C_6$]permethrin and *trans*-[$^{13}C_2$]DCCA were added to the matrix before the microbeads step. Then, 1 g (or mL) of the matrix (except urine), containing the target analytes and their internal standards, was treated by a methanolic/hydrochloric acid solution. This step ensures the derivatization of the metabolites and the cleavage of the conjugated metabolites. The methanolic/hydrochloric acid solution has no impact on the recovery of permethrin, as pointed out by Lestremau et al. (2014). The different compounds were then extracted with toluene. The extraction of permethrin in fat was performed with an acetonitrile/dichloromethane solution containing a purification step with a blend of Strata X-AW and Na_2SO_4. A column ZB-5MS (30 m × 0. 25 mm I.D., 1 μm) and a Varian gas chromatograph 3800 coupled with a Varian ion trap mass spectrometer 4000 were used. The limits of quantification were 50 μg/L for *cis*- and *trans*-permethrin, and 25 μg/L for *cis*- and *trans*-DCCA. When a compound was detected in a sample at a concentration below the limit of quantification (LOQ), the compound concentration was set to LOQ/2. This approach is quite widely used in toxico/phamacokinetic studies (Beal, 2001; Soucy et al., 2006).

Both parent compounds and metabolites DCCA, 3-PBA and 4′-OH-PBA were quantified in urine by LC–MS/MS (Le Grand et al., 2012). A column Atlantis T3 (150 mm × 2.1 mm I.D., 5 μm) was required with Shimadzu LC-10 AD pumps coupled with a mass spectrometric AB