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Documenting the kinetic time course of lambda-cyhalothrin metabolites in orally exposed volunteers for the interpretation of biomonitoring data

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ABSTRACT

Lambda-cyhalothrin is a pyrethroid pesticide largely used in agriculture. Exposure assessment can be performed by measuring key urinary metabolites. For a proper use of biomonitoring data, it is however important to gain information on the toxicokinetics of these key biomarkers of exposure. A human volunteer study was performed to document the plasma and urinary time courses of major lambda-cyhalothrin metabolites. Seven volunteers ingested 0.025 mg kg^{-1} body weight of lambda-cyhalothrin. Blood samples were withdrawn prior to dosing and at fixed time periods over the 72 h-period following ingestion and complete urine voids were collected pre-exposure and at pre-established intervals over 84 h post-dosing. The cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid (CFMP) and 3-phenoxybenzoic acid (3-PBA) metabolites were quantified in these samples. Plasma concentrations of CFMP and 3-PBA increased rapidly after ingestion, with average peak values at 3.1 and 4.0 h post-dosing, respectively; subsequent elimination phase showed a rapid decay with a mean half-life ($t_{½}$) of ≈5.3 and 6.4 h for CFMP and 3-PBA, respectively. Urinary rate time courses displayed a profile similar to the plasma concentration-time curves with corresponding mean $t_{½}$ of ≈4.2 and 5.9 h. In the 84-h period post-treatment, on average 21% of lambda-cyhalothrin dose were excreted in urine as CFMP as compared to 30% as 3-PBA. Overall, CFMP and 3-PBA metabolites were confirmed to be major metabolites of lambda-cyhalothrin and exhibited similar kinetics with short half-lives; they thus both appear as useful biomarkers of exposure to lambda-cyhalothrin in humans.

1. Introduction

Pyrethroids are a group of synthetic insecticides, which were initially manufactured in the 1970s following the ban of organochlorine insecticides. They are analogs of the naturally occurring compound pyrethrin derived from the flowering plants *Chrysanthemum cinerariae folium*. They are basically divided into two types: type I without a cyano moiety at the alpha-position of the cyclopropane carboxylate structure and type II having an alpha-cyano moiety (Nasuti et al., 2003).

The mode of action of pyrethroids on the neurological system of insects is known to be via interactions with neuronal voltage-gated sodium channels (Field et al., 2017). Pyrethroids bind to these channels and prevent their transition from an activated to an inactivated state (Field et al., 2017). This causes a prolonged depolarization of excitable cell membranes leading to paralysis and death of targeted insects (Narahashi et al., 1992, 2007). Pyrethroids are much less neurotoxic to humans, owing in part to differences in sodium channel sequences, and thus in the binding capacity of pyrethroids to these channels (Field et al., 2017). However, there have been reports of acute symptoms after pyrethroid poisoning or incidents in workers; these include neurological and respiratory symptoms (Saillenfait et al., 2015). Some epidemiological studies have also found associations between pyrethroid exposure and effects on male human reproduction (sperm sex ratio) as well as child neurodevelopment (Jurewicz et al., 2016; Watkins et al., 2016). Pyrethroids have also been reported to induce modulations of the endocrine and immune systems as well as oxidative stress following high dosing in rats (Righi et al., 2009; Yousef, 2010; Ansari et al., 2012; Fetoui and Gdoura, 2012). Nevertheless, transposition of the latter results for risk assessment in humans exposed to much lower doses, repeated over time, has not been established.

Exposure to pyrethroids in the general population may occur by dietary intake of pesticide residues on fruits, vegetables, cereals but also by dermal contact and respiratory uptake in certain circumstances (Fortin et al., 2009; Curwin et al., 2005). In agricultural workers,

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respiratory exposure of applicators has been documented as well as dermal exposure of field crop workers, but indirect oral exposure by hand-to-mouth contact has also been reported (HSE, 2007; Ratelle et al., 2016; Ferland et al., 2015).

Because of their large use, several *in vitro* metabolism studies and *in vivo* experiments in mammalians have been carried out for different pyrethroids to clarify their common metabolic pathways (Abe et al., 2017). The main metabolic pathways were found to be oxidations of both acid and alcohol moieties, ester cleavage, and conjugation reactions (Kaneko, 2010; Kaneko and Miyamoto, 2001; Mikata et al., 2012; Wang et al., 2016).

The metabolism of lambda-cyhalothrin, which is among the most widely applied pyrethroid in agriculture, has been documented to occur by hydrolysis catalyzed by esterases (Anadón et al., 2006). The main degradation metabolites include cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2 dimethylcyclopropanecarboxylic acid (CFMP) and 3-phenoxybenzoic acid (3-PBA) (Chester et al., 1992). The former metabolite is specific to lambda-cyhalothrin while the latter is common to other pyrethroids. These metabolites have been measured in human urine as biomarkers of exposure (Thomas et al., 2016). In particular, Chester et al. (1992) measured levels of lambda-cyhalothrin metabolites in spot urine samples of workers exposed to this pesticide.

However, until now, data on the kinetic behavior of lambda-cyhalothrin in humans are scarce. The only available biokinetic information is based on animal experiments. Anadón et al. (2006) published data on the kinetics of the lambda-cyhalothrin, which is limited to the rats and the fate of the parent compound in the body. Knowledge of the kinetics of metabolites is essential for a proper use of these biomarkers of exposure in biomonitoring studies. Therefore, the aim of this study was: i) to document the time courses of lambda-cyhalothrin metabolites in humans after oral exposure, and ii) to determine main toxicokinetic parameters to help interpretation of biomonitoring data in workers exposed to this pesticide.

2. Materials and methods

2.1. Study design

A controlled kinetic time course study of lambda-cyhalothrin metabolites in accessible biological matrices of healthy subjects was conducted following an acute oral exposure. A preliminary study was initially conducted on one volunteer at a dose equal to the acute oral reference dose (RfD) of 0.0025 mg/kg established by the U.S. EPA (U.S. EPA, 2004). Metabolites were measured in plasma and urine samples prior to exposure and at predetermined time points post-treatment. Since this dosing level did not allow to clearly establish the plasma kinetics, dosage was increased to 0.025 mg/kg for the main kinetic study.

The experimental protocol and consent forms were approved by the Research Ethics Committee of the University of Montreal (certificate 15-085-CERES-P). All the participants gave their written consent and were informed of the risks of participating and their right to withdraw from the study at any time. The participants received a monetary compensation for their time and any inconvenience caused. None of the volunteers reported symptoms during the study period.

2.2. Subjects

Participants were recruited on a voluntary basis among students of the University of Montreal and their family and friends. Volunteers were young healthy, non-smokers and did not take medication. They had not been exposed to pyrethroids during the three month-period prior to the experimental dosing protocol, except perhaps through diet. During the three days prior to the onset of the study, participants were requested not to eat fruits, vegetables or nuts and were provided with organic bread, cereals, milk and jelly. During the 4-day biomonitoring period, volunteers were provided with the meals and snacks prepared with certified organic ingredients in order to limit ingestion of contaminated food, since it is known that diet is usually the main absorption route of pesticides in the general population (Schettgen et al., 2002). They were also requested to avoid consumption of tea, herbal tea, alcohol and drugs during this period, since it can affect metabolism of some chemical compounds by interfering with enzyme activity (Choi et al., 2002; Gueguen et al., 2006).

2.3. Dosing

The experimental dosing and sampling were conducted at the Department of Environmental and Occupational Health of the University of Montreal, Canada. The morning of study onset, a control blood sample was collected from each volunteer 30 min before dosing and a complete urine void was collected during the three hours prior to dosing.

A preliminary acute oral dosing study was conducted in one volunteer to verify if metabolite levels were sufficiently high to establish a kinetic time course in plasma and urine. For this preliminary experiment, the dosing was set at 0.0025 mg of lambda-cyhalothrin/kg body weight (bw) (purity ≤ 100%; purchased at Supelco, Sigma-Aldrich, Oakville, Ontario), the value of the acute RfD. For the main kinetic time course study in orally exposed volunteers, the dosing level was increased to 0.025 mg/kg bw of lambda-cyhalothrin to be able to establish a kinetic time course in plasma. Seven other volunteers participated in this main kinetic study. The compound administered was dissolved in biological olive oil (1 mg of product in 2 ml of oil for the 0.0025 mg/kg bw dosing and 10 mg of product in 2 ml of oil for the 0.025 mg/kg bw dosing). Following dosing, volunteers were given 100 ml of water. The tip used for dosing was then rinsed with pure oil and administered to the participants with another 100 ml of water.

2.4. Sampling for oral exposure

For oral exposure, blood samples of 30 ml (3 × 10 ml tubes) were collected at specific times, up to 72 h post-dosing: −0.5, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 24, 48, and 72 h after ingestion (n = 12 samples per individual per dosing). To facilitate collection, a catheter was installed by a nurse prior to dosing. Blood was withdrawn by the nurse into vacutainers with a code, the date and time of sampling. Immediately after collection, blood samples were centrifuged to precipitate red blood cells and isolate plasma. Plasma samples were then split into labelled aliquots and stored at −20 °C until analysis.

Total urine voided over specific periods was also collected: −3–0, 0–3, 3–6, 6–9, 9–12, 12–24, 24–36, 36–48, 48–60, 60–72, 72–84 h post-administration (n = 11 collections per individual). To assist participants, they received a schedule specifying the date and the required time of urine collection. They then only had to complete the sheet with the actual time of collection and to indicate whether or not there were any urine losses. Each timed-void was collected in separate polypropylene Nalgene® bottles of 500 ml, already identified. Once collected, urine samples were kept in the refrigerator prior to measurement of total urine volume per void. Each urine collection then was aliquoted in 3 labelled pyrex tubes of 15 ml and one bottle of 90–100 ml before storage at −20 °C until analysis.

Volunteers spent the first 12-h postdosing in a room at the Department. During the following 4 days, participants visited the Department for their daily morning blood sampling and to hand-in urine collections. Volunteers were further asked to fill a questionnaire to document personal information (weight, height) and life habits (i.e. physical activities, exposure to second-hand smoke) during the study period as well as confirm the absence of consumption of drugs, alcohol, tobacco or non-organic food during this period, and possible dosing-related symptoms.