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In vitro assessment of pyrethroid bioaccessibility via particle ingestion

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ABSTRACT

Due to their intensive use in agricultural and residential pest control, human exposure to residues of multiple pyrethroids frequently occurs. Pyrethroids have exceptionally high affinity for solid particles, highlighting the need to understand human exposure through oral ingestion of contaminated soil or dust particles. In this study, we used artificial gastrointestinal fluids to measure the desorption or bioaccessibility of eight current-use pyrethroids in soil and dust samples. Tenax was further included as a sink in parallel treatments to simulate the effect of removal due to transfer of pyrethroids to lipid membranes. The use of 0.4 g of Tenax in 20 mL digestive fluids resulted in rapid and efficient trapping of pyrethroids, and further, greatly increased bioaccessibility. In the artificial digestive fluids without Tenax, 6.0–48.0% of pyrethroids were desorbed over 21 h, and the fractions increased by 1.6–4.1 folds to 21.5–79.3% with the Tenax sink. Therefore, 6.0–79.3% of soil or dust-borne pyrethroids may be considered bioavailable upon ingestion. While protein and sucrose increased the estimated bioaccessibility, co-presence of lipid (vegetable oil) decreased the bioaccessibility of pyrethroids, likely due to competitive phase partition. Pyrethroids were also found to be unstable in the artificial intestinal fluid containing pancreatin, further decreasing the potential bioaccessibility of pyrethroids on soil or dust particles. The limited bioaccessibility should be considered to refine the prediction of human exposure and risk through oral ingestion of pyrethroid residues.

1. Introduction

Pesticides, such as synthetic pyrethroids, are widely used to manage insect pests in agriculture as well as to protect public health (Amweg et al., 2005; Bradberry et al., 2005). It was estimated that pyrethroids account for about 35% of the global insecticide market share (Global Pyrethroid Insecticide Market, 2017). Much of pyrethroid use happens in and around residential homes to eradicate structural and vector pests. Consequently, residues of multiple pyrethroids have been found in dust particles on outdoor surfaces in residential areas (Jiang et al., 2016; Richards et al., 2016), in wipe samples collected from public and residential floors (Julien et al., 2008; Stout et al., 2009; Tulve et al., 2006), and in indoor dust (Morgan et al., 2007; Quiros-Alcala et al., 2011; Starr et al., 2008). Monitoring for pyrethroid metabolites in urine samples showed widespread human exposure to pyrethroids in the general population (Barr et al., 2010; Lu et al., 2006; Naeher et al., 2010; Oulhote and Bouchard, 2013). Residues of pyrethroids were also detected in human breast milk in urban and rural regions of different countries (Corcellas et al., 2012; Feo et al., 2012; Heudorf and Angerer, 2001; Sereda et al., 2009). There is a growing concern about the possible effects of pyrethroids on immunological and neurobehavioral development, respiratory health, and reproductive function of adult humans (Costa et al., 2013; Jurewicz et al., 2015; Meeker et al., 2009; Saillenfait et al., 2015). Exposure to pyrethroids has also been identified as one of the risk factors for the development of childhood brain tumors (Chen et al., 2016; van Maele-Fabry et al., 2017). Therefore, the widespread occurrence and potential human health risks dictate a better understanding of human exposure to this class of insecticides.

Ingestion of soil and dust is a major route for human exposure to pyrethroids because of their strong affinity for particles, and this risk may be particularly important for children due to their frequent hand-to-mouth activities (Moya et al., 2004; van Maele-Fabry et al., 2011). The traditional risk assessment used the total chemical concentration (C_{Total}) for exposure calculation; however, for strongly hydrophobic organic compounds (HOCs) such as pyrethroids, this may result in overestimated risks (Costera et al., 2009; Rostami and Juhasz, 2011). Strong sorption to organic matter in soil or dust particles may lead to slow desorption and hence reduced bioavailability in the gastrointestinal tract (Feo et al., 2010; Laskowski, 2002). Therefore, bioavailability is a critical variable for understanding the risk of pyrethroids

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via oral ingestion.

In vivo methods using swine or rat animal models are preferred as they offer realistic bioavailability assessment (Duan et al., 2014; James et al., 2011; Juhasz et al., 2014; Rostami and Juhasz, 2011). However, ethical concerns, high costs and low sample through-put make this approach impractical for routine testing (Cui et al., 2016). Several *in vitro* physiologically based extraction methods have been developed to evaluate bioaccessibility of HOCs associated with soil or dust (Abdallah et al., 2012; Dean and Ma, 2007; Tilston et al., 2011). In the application of such *in vitro* methods, however, the bioaccessibility of HOCs may be incorrectly estimated, because actual mammalian digestion systems contain lipid membranes that act to continuously transfer HOCs out of the digestive fluid (James et al., 2011; Juhasz et al., 2016; Fang and Stapleton, 2014). To simulate this removal process, several materials, including C18 membrane, silicon rod, and Tenax, were introduced as a sorption sink to improve the prediction (Gouliarmou et al., 2013; James et al., 2011; Juhasz et al., 2016; Li et al., 2015; Zhang et al., 2015). However, despite their wide occurrence, to date the bioavailability of particle-borne pyrethroid residues for human exposure is poorly understood.

In this study, we coupled colon extended physiologically based extraction test (CE-PBET) (Gouliarmou et al., 2013) with Tenax as an absorption sink to assess oral bioaccessibility of pyrethroids in dust and soil samples. The results from this study may shed light on the bioaccessibility of pyrethroid residues on soil or dust particles, and the developed method may be adopted for high throughput screening to improve our understanding of bioavailability-based human exposure to pyrethroid insecticides.

2. Materials and Methods

2.1. Chemicals

Individual standards of pyrethroids (fenpropathrin, lambda-cyhalothrin, bifenthrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate, and deltamethrin) with a purity of ≥97.0% were obtained from FMC (Princeton, PA), Bayer CropScience (Kansas City, KS), Syngenta (Greensboro, NC) and Valent (Mahomet, IL). Isotope-labeled bifenthrin-d_5 (99%) and phenoxy-$^{13}C_6$-*cis*-permethrin (99%) were purchased from Toronto Research Chemicals (North York, Ontario, Canada) and Cambridge Isotope Laboratories (Andover, MA), respectively. A stock solution containing all 8 pyrethroids was made in acetone at 10 mg/L for each compound. Tenax TA resin (60–80 mesh) was purchased from Scientific Instrument Services (Ringoes, NJ). The simulated gastric, intestinal, and colon fluids were prepared according to Tilston et al. (2011). All solvents and other chemicals used in the study were of gas chromatography (GC) or analytical grade.

2.2. Soil and dust samples

Five soil and one dust samples were used in the bioaccessibility assessment. The soil samples were collected from Orange County (denoted as OC1 and OC2), City of Riverside (RS1 and RS2), and Salton Sea (SS1) in California. The dust sample (OC3) was collected from outdoor pavement in an Orange County residential area using a handheld vacuum. Both soil and dust samples were air-dried and passed through a 250-μm sieve before use. Preliminary experiments showed that the dust sample contained various pyrethroids and was directly used for bioaccessibility measurement, while the soil samples were spiked with the test compounds prior to use. Briefly, aliquots of 10 g (dry weight) of soil were spiked with 0.2 mL pyrethroid stock solution in acetone. After the carrier solvent was evaporated, the spiked samples were mixed at 30 rpm on a roller at room temperature for 7 d to attain homogenous distribution and a short-term aging effect. Prior to bioaccessibility assessment, the total concentrations of pyrethroids (C_{Total}) in the soil and dust samples were measured according to Richards et al. (2016) (Table S1). The organic carbon (OC) contents of the samples were determined on a Flashea 1112 series N/C Analyzer (Thermo Electron, Waltham, MA) (Table S1).

2.3. Tenax sorption experiments

To assess the feasibility of Tenax as an absorptive sink in CE-PBET, the sorption kinetics and efficiency of pyrethroids on Tenax beads were evaluated in the simulated intestinal and colon fluids through preliminary experiments. Briefly, aliquots of the intestinal or colon solution (20 mL) were transferred to 50-mL glass tubes and maintained at 37 °C in a water bath. The fluids were spiked with pyrethroid mixtures to achieve an initial concentration of 100 μg/L for each compound. The fluids together with Tenax beads (0.1, 0.2 or 0.4 g) were then incubated at 37 °C on a Model 400 Hybridization Incubator at 20 rpm (SciGene, Sunnyvale, CA). For the experiment with intestinal fluid, triplicate samples were withdrawn after 5, 15, 30, 60, 120, and 240 min of equilibration. The samples were filtered through a Whatman glass microfiber filter (Whatman, Maidstone, UK) to recover the Tenax beads. The recovered Tenax beads were rinsed with deionized water, air-dried, transferred to a 20-mL glass vial, and extracted by sonication using 5 mL acetone-hexane (1:1, v/v) for 5 min. The extraction was repeated three consecutive times. The extracts were combined in a test tube, concentrated under N_2 to near dryness and reconstituted in 1.0 mL of hexane. For the colon fluid experiment, similar procedures were followed except that the incubation time was prolonged to 960 min to simulate the physiological residence time of the fluid in colon.

The sorption kinetics of pyrethroids by Tenax may be described using the following equation (Li et al., 2016):

$$C_t = C_0 \cdot F_{eq}(1 - e^{-kt}) \quad (1)$$

where C_t is the pyrethroid concentration (μg/L) sorbed on Tenax at time t (min), C_0 is the initial pyrethroid concentration (μg/L) in the CE-PBET solution, F_{eq} is the fraction of pyrethroid sorbed at equilibrium, and k is the rate constant (1/min).

2.4. Bioaccessibility measurement using Tenax sorptive CE-PBET

After the assessment of Tenax as an absorptive sink in CE-PBET, Tenax assisted CE-PBET was applied to measure the bioaccessibility of pyrethroids in the soil and dust samples. Because bile salts may reduce the surface tension and cause precipitation of Tenax beads in simulated gastrointestinal fluids (Fang and Stapleton, 2014; Li et al., 2016), Tenax beads were enclosed in a 100-mesh stainless steel insert during the exposure to facilitate their recovery (Fig. S1). A preliminary test showed that the insert did not affect the uptake of pyrethroids by Tenax beads. The general procedure of bioaccessibility measurement followed that in Tilston et al. (2011). Briefly, 50-mL glass centrifuge tubes containing 0.2 g of soil or dust sample, 0.4 g of Tenax beads, and 20 mL of gastric fluid (pH 2.5) were incubated at 37 °C and 20 rpm. After 1 h of incubation, the gastric fluid was altered to intestinal fluid by adjusting pH to pH 7.0 and addition of 0.035 g bile salts and 0.01 g pancreatin. After 4 h of incubation, the Tenax insert was removed and the test substrate was recovered by centrifugation at 670 g for 10 min. The recovered substrate and Tenax insert along with 20 mL of the colon fluid were added back to the tubes and the mixture was further incubated at 37 °C for 16 h to mimic the colon compartment. After the final incubation, the Tenax insert was removed and analyzed for pyrethroids.

For comparison, treatments without Tenax were included and incubated under the same conditions. After incubation, the supernatants of intestinal fluid and colon fluid were combined and extracted by liquid-liquid extraction. Briefly, the combined fluid was extracted with 10 mL dichloromethane in a 250-mL separatory funnel for three consecutive times. The extracts were passed through anhydrous sodium sulfate and combined. The combined extracts were concentrated under