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In vitro human metabolism of permethrin isomers alone or as a mixture and the formation of the major metabolites in cryopreserved primary hepatocytes

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ABSTRACT

In vitro metabolism of permethrin, a pyrethroid insecticide, was assessed in primary human hepatocytes. *In vitro* kinetic experiments were performed to estimate the Michaelis–Menten parameters and the clearances or formation rates of the permethrin isomers (*cis*- and *trans*-) and three metabolites, *cis*- and *trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis*- and *trans*-DCCA) and 3-phenoxybenzoic acid (3-PBA). Non-specific binding and the activity of the enzymes involved in permethrin's metabolism (cytochromes P450 and carboxylesterases) were quantified. *Trans*-permethrin was cleared more rapidly than *cis*-permethrin with a 2.6-factor (25.7 ± 0.6 and 10.1 ± 0.3 μL/min/10^6 cells respectively). A 3-factor was observed between the formation rates of DCCA and 3-PBA obtained from *trans*- and *cis*-permethrin. For both isomers, the rate of formation of DCCA was higher than the one of 3-PBA. The metabolism of the isomers in mixture was also quantified. The co-incubation of isomers at different ratios showed the low inhibitory potential of *cis*- and *trans*-permethrin on each other. The estimates of the clearances and the formation rates in the co-incubation condition did not differ from the estimates obtained with a separate incubation. These metabolic parameters may be integrated in physiologically based pharmacokinetic (PBPK) models to predict the fate of permethrin and metabolites in the human body.

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1. Introduction

Permethrin, a synthetic pyrethroid insecticide, is widely used under different forms in house treatment and agriculture (Stout et al., 2009; US EPA, 2011). As a consequence, humans are frequently exposed to this insecticide, and this was confirmed by the detection of urinary permethrin metabolites in children and adults in biomonitoring studies (Barr et al., 2010; Ueyama et al., 2010). Permethrin is suspected to induce neuronal disturbances such as paresthesia or headaches (Bradberry et al., 2005) and was also associated to modifications of the human semen quality (Meeker et al., 2008; US EPA, 2011; Young et al., 2013; Imai et al., 2014). Since the active form is the parent compound, the quantification of metabolism, the only route of elimination from the circulatory system, is required to assess the internal exposure in humans.

The metabolic pathway of the two isomers *cis* and *trans* of permethrin in mammals was previously identified by Gaughan et al. (1977). In humans, permethrin can be hydrolyzed by carboxylesterases (CE) or oxidized by cytochromes P450 (CYP) directly into the metabolites *cis*- or *trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis*- and *trans*-DCCA), specific to each isomer, and into the (un)oxidized form of 3-phenoxybenzoic alcohol (3-PBAlc) (Crow et al., 2007). Several steps of oxidation by alcohol or aldehyde desydrogenases occur and lead to obtain the final metabolite 3-phenoxybenzoic acid (3-PBA) common to both isomers (Choi et al., 2002). DCCA and 3-PBA can finally be conjugated by phase II enzymes (Takaku et al.,

Abbreviations: ADH, alcohol deshydrogenase; ALDH, aldehyde deshydrogenase; CE, carboxylesterase; *cis*-p, *cis*-permethrin; CYP, cytochrome P450; DCCA, 3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid; FBS, fetal bovine serum; PNP, 4-paranitrophenol; PNPA, 4-paranitrophenol acetate; 3-PBA, 3-phenoxybenzoic acid; 3-PBAlc, 3-phenoxybenzoic alcohol; PBS, phosphate buffered saline; *trans*-p, *trans*-permethrin.

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2011; Mikata et al., 2012). The metabolites DCCA and 3-PBA are commonly used as biomarkers of exposure to permethrin and other pyrethroids in biomonitoring studies. Fig. 1 represents a simplified metabolic pathway with the involved enzymes.

Metabolism in humans is usually predicted using *in vitro* models coupled to methodologies for *in vitro*/*in vivo* extrapolation (Lave et al., 1999; Obach, 1999; Hallifax et al., 2005; Riley et al., 2005; Rostami-Hodjegan and Tucker, 2007). The main sites of metabolism for permethrin and the enzymes involved in humans have been established using microsomes and recombinant cells expressing CYPs and CEs (Nishi et al., 2006; Ross et al., 2006; Crow et al., 2007; Scollon et al., 2009; Yang et al., 2009; Takaku et al., 2011; Lavado et al., 2014). In humans, the liver is the major site of metabolism of permethrin with the highest abundance of esterases and CYPs (Crow et al., 2007). In their study, Scollon et al. (2009) quantified the hepatic metabolic rates for *cis*- and *trans*-permethrin using microsomes including the hydrolysis and the oxidation pathways. *Trans*-permethrin was eliminated faster than *cis*-permethrin (by a 12-factor) and reduced metabolic rates (about a 2-factor) during the co-incubation of the two isomers were observed suggesting an interaction between the *cis*- and *trans*-permethrin. Since the mixture of the two isomers is suspected to increase the residence time of the active compound (here the parent compound) in the body and therefore the associated risk, the interaction between the two isomers needs to be characterized more deeply to be used in risk assessment. Indeed humans are exposed to mixtures of permethrin isomers in which the ratio between *cis*- and *trans*-isomers varies with time and may depend on the exposure sources (Eadsforth and Baldwin, 1983; Woollen et al., 1992; IPCS, 1994; ATSDR, 2003).

In vitro metabolic rates provide valuable and quantitative knowledge that can be extrapolated to *in vivo* situations and then be integrated in toxicokinetic models to simulate the fate of the compound in the human body. For non-persistent compounds such as pyrethroid insecticides, the metabolic clearances are model parameters that highly influence their internal levels in the body. Indeed, sensitivity analyses performed on a physiologically based pharmacokinetic (PBPK) model developed for permethrin in humans (Wei et al., 2013) showed that the hepatic intrinsic clearance was one of the most influential parameters for the blood concentration under several exposure scenarios, together with physiological parameters related to the absorption of the compound. The knowledge of the metabolic clearances is therefore a critical issue for the development of toxicokinetic models. The quality of the *in vitro*/*in vivo* extrapolation of metabolic rates relies mainly on the choice of the *in vitro* model for the compounds of interest, the experimental settings and the analysis of the kinetic data (McMullin et al., 2007; Pelkonen and Turpeinen, 2007). Several *in vitro* models are preferably used to derive the metabolic rates such as microsomes, liver slices or primary hepatocytes (Lipscomb and Poet, 2008). The use of primary hepatocytes which closely reflect *in vivo* conditions by containing all the physiological factors (Hewitt et al., 2007; Yoon et al., 2012) has already been proven to increase the quality of predictions of the hepatic clearance for several drugs (Brown et al., 2007b). In the case of permethrin, the cytosolic fraction of hepatocytes also contains esterases that could participate to metabolism (Crow et al., 2007). Cryopreserved hepatocytes are now routinely used since their activity and stability are similar to fresh hepatocytes (McGinnity et al., 2004). Other experimental conditions like the addition of serum in the medium to mimic *in vivo* equilibrium (Blanchard et al., 2004; Li, 2007) and evaluation of non specific binding, especially for lipophilic compounds such as permethrin, have to be considered to improve the quality of the extrapolation (Ouattara et al., 2011; Groothuis et al., 2013) .

In the present study, we propose to determine in adequate conditions for *in vitro*/*in vivo* extrapolation the Michaelis–Menten parameters of the depletion of *cis*- and *trans*-permethrin and the effect of co-incubation of both isomers on human primary hepatocytes. The rates of formation of metabolites *cis*- and *trans*-DCCA and 3-PBA, biomarkers of exposure to permethrin, will be also estimated. The depletion and formation rates will be extrapolated to *in vivo* rates in order to be used for the calibration of toxicokinetic models.

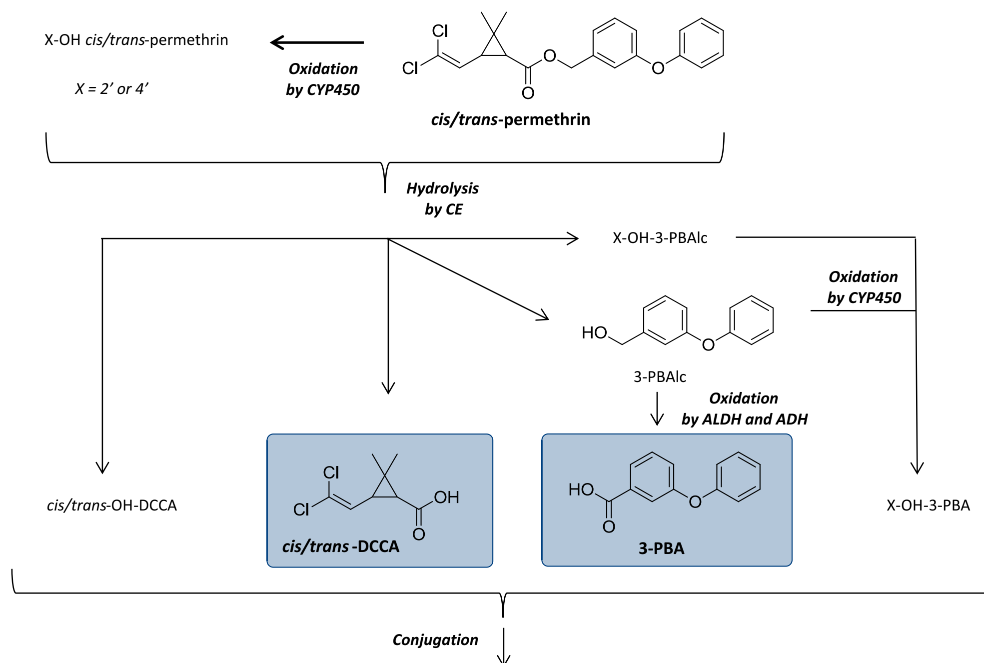

Fig. 1. A simplified representation of the pathways of metabolism of *cis*-permethrin and *trans*-permethrin into the metabolites *cis*/*trans*-DCCA and 3-PBA commonly dosed as biomarkers of an exposure in biomonitoring studies. Permethrin is hydrolyzed and/or oxidized. DCCA is a direct metabolite of the hydrolysis of permethrin. The formation of 3-PBA needs the hydrolysis of permethrin into 3-PBAlc followed by its oxidation. Derived from Scollon et al. (2009), Ross et al. (2006), Choi et al. (2002), Takaku et al. (2011).

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