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Estimating the cumulative human exposures to pyrethroids by combined multi-route PBPK models: Application to the French population

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Abstract

Human biomarkers of exposure to pyrethroid insecticides are usually urinary concentrations of metabolites that can be specific to a pyrethroid or common to several compounds. We developed a global toxicokinetic model that links the external exposure to four widely-used pyrethroids and their isomers (deltamethrin and *cis* and *trans* isomers of permethrin, cypermethrin, and cyfluthrin) to the urinary concentrations of metabolites (*cis*- and *trans*-DCCA, 3-PBA, F-PBA and DBCA). This global model includes physiologically based pharmacokinetic models for each parent compound and one-compartment models for the metabolites. Existing *in vivo*, *in vitro* and *in silico* data were used for model calibration, and human toxicokinetic data for model evaluation. Overall, the global model reproduced the data accurately as about 90% of predictions were inside the 3-fold error interval. A sensitivity analysis showed that the most influent parameter for each urinary metabolite concentration was the fraction of parent compound that is transformed into that metabolite. The global model was then tested with realistic exposures for the French population: the predictions were consistent with biomonitoring data. The global model is a tool that will improve the interpretation of biomonitoring data for pyrethroids.

Keywords: PBPK model, pyrethroids, metabolites, cumulative exposure, aggregate exposure, biomonitoring

1   Introduction

31

32 Pyrethroids are a family of organic compounds that have numerous usages as pesticides and biocides.

33 They were introduced on the market in the mid-1970s and are increasingly used since the 1990s due to

34 their broad spectrum (Corbel and N'Guessan, 2013; Feo et al., 2010; Williams et al., 2008). Many

35 biomonitoring studies have shown the wide exposure of the general population in several countries

36 (Barr et al., 2010; Heudorf et al., 2006; Morgan, 2012; Ueyama et al., 2010). In France, biomonitoring

37 studies showed that the adult population is exposed to higher levels than the German or North American

38 populations (Dereumeaux et al., 2018; Fréry et al., 2011). Biomarkers of exposure that are used to

39 estimate the population exposure are the urinary concentrations of five metabolites: 3-phenoxybenzoic

40 acid (3-PBA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-

41 DCCA and *trans*-DCCA), 4-fluoro-3-phenoxybenzoic acid (F-PBA), 3-(2,2-dibromovinyl)-2,2-dimethyl

42 cyclopropane carboxylic acid (DBCA). These biomarkers can be specific to a pyrethroid parent

43 compound or common to several pyrethroids (Ueyama et al., 2010). For example, DBCA is specific to

44 deltamethrin and *cis*-DCCA and *trans*-DCCA are common to several pyrethroids (*cis*- and *trans*-

45 permethrin, *cis*- and *trans*-cyfluthrin and *cis*- and *trans*-cypermethrin). Common metabolites therefore

46 reflect a cumulative exposure to several pyrethroid compounds.

47 Toxicokinetic models for specific pyrethroids have been developed in humans to relate external

48 exposure to internal metabolite or parent compound concentrations by describing the process of

49 absorption, distribution, metabolism and excretion (ADME). These models can be multi-compartmental

50 (Cote et al., 2014; Xue et al., 2014) or physiologically based pharmacokinetic models (PBPK) (Darney

51 et al., 2018; Godin et al., 2010; Tornero-Velez et al., 2012; Wei et al., 2013) that can facilitate the

52 extrapolation between individuals and between exposure scenarios (Andersen et al., 1995). Human

53 PBPK models for deltamethrin (Godin et al., 2010) and permethrin (Darney et al., 2018; Tornero-Velez

54 et al., 2012; Wei et al., 2013) are currently available. These models were developed based on *in vivo*

55 experiments in rats and then extrapolated to humans using *in vitro* data (Godin et al., 2006; Hedges et

56 al., 2018; Scollon et al., 2009; Willemin et al., 2015). The PBPK models for permethrin were applied to

57 predict the levels of urinary metabolites using exposure scenarios including several sources of exposure.

58   For instance, Tornero-Velez et al. (2012) and Darney et al. (2018) applied their PBPK model for

59   permethrin to predict the urinary concentrations of the DCCA metabolite in the US and French

60   populations and compared the predictions to biomonitoring data. Similarly, Wei et al. (2013) assessed

61   the permethrin exposure of flight attendants. These modeling works studied the link between the

62   exposure to one pyrethroid (or a combination of the isomers of one pyrethroid) and the urinary

63   concentration of only one metabolite.

64   The interpretation of the urinary levels of common metabolites measured in populations requires to go a

65   step further in order to account for the cumulative exposure to several pyrethroids compounds together

66   with the aggregate exposure from the different routes. Indeed it is particularly important to determine

67   which part of the common metabolite is attributable to a pyrethroid, as the toxicity levels differ between

68   pyrethroids (ATSDR, 2003). Recently Aylward *et al.* (2018) first attempted to integrate such

69   information in the derivation of biomonitoring reference values for the urinary 3-PBA metabolite for

70   risk assessment. They developed a 2-tier approach whose second tier accounts for the weight of the

71   relative exposures to several pyrethroid compounds and for the differences in toxicity magnitude.

72   In this paper, we develop a global toxicokinetic model to predict the urinary levels in humans of four

73   pyrethroid metabolites, *i.e. cis-* and *trans*-DCCA, F-PBA and DBCA, following exposures to

74   permethrin, cypermethrin, cyfluthrin and deltamethrin. The 3-PBA metabolite was also included in the

75   global model but not all its parent compounds were taken into account. A generic PBPK model was

76   adapted to the toxicokinetics of these pyrethroid compounds (Beaudouin et al., 2010), and one-

77   compartment models were developed for the metabolites. The models for the parent compounds and the

78   metabolites were linked together. A total of seven parent compounds (*cis* and *trans* isomers of

79   permethrin, cypermethrin, cyfluthrin, plus deltamethrin), and a total of 5 metabolites were included in

80   the global model. This latter was evaluated before being applied to the estimation of the exposure of the

81   French adult population. The cumulative and aggregated exposure to the four pyrethroids was calculated

82   using national data, including exposure from food, air and dust. The model predictions of the urinary

83   levels were compared to the biomonitoring data of the National Health Nutrition Study (Fréry et al.,

84   2011) for men and women. Finally, a sensitivity analysis was performed to identify the model

4

85 parameters that influence the internal concentrations of parent compounds and the urinary concentration

86 of metabolites in this context of multi-routes and multiple compounds.

2 Materials and Methods

2.1 Chemicals

89 Permethrin, cypermethrin and cyfluthrin are combinations of *cis* and *trans* isomers whereas

90 deltamethrin is a pure *cis*-isomer. A total of 7 parent compounds was then studied in our work. All these

91 pyrethroids undergo extensive metabolic transformations to form several chemical species. Table 1

92 presents the parent compounds with their respective urinary metabolites that are usually measured in

93 biomonitoring studies. Among the five metabolites, only DBCA is specific to one pyrethroid, namely

94 deltamethrin (DLT). F-PBA is specific to cyfluthrin but can be formed from the two isomers. *Cis*- and

95 *trans*-DCCA can be formed respectively from the *cis* and *trans* isomers of permethrin (*cis*-PM and

96 *trans*-PM), cypermethrin (*cis*-CYP and *trans*-CYP) and cyfluthrin (*cis*-CYF and *trans*-CYF). The

97 metabolite 3-PBA is common to the four pyrethroids and their isomers, and can also be generated from

98 other pyrethroids (Starr et al., 2008).

2.2 Model structure for the parent compounds

100 A generic PBPK model, previously developed by Beaudouin et al. (2010), was used to describe the

101 toxicokinetics of the pyrethroids in humans. This model is based on a detailed description of the

102 anatomy of the human body and comprises 23 tissue compartments. The model structure is similar for

103 men and women. Several changes were made to account for the specificities of pyrethroids (Figure 1).

104 In the following, the quantities are in mg, the concentrations in mg/L, the volumes in L, and the blood

105 flows in L/h.

106 Ingestion and inhalation were the exposure routes included in the initial model. Dermal contact was then

107 added. Dermal absorption was modeled as a one-directional diffusive process across the stratum

108 corneum and the viable epidermis. The skin compartment of the initial model was subdivided into two

109 compartments representing the stratum corneum that is in contact with the skin's surface, and the

110   vascularized tissue (named *skin* in the following). The transfer of chemicals between the different layers

111   is modelled by diffusion processes (Tornero-Velez et al., 2012):

112
$$\frac{d(Q_{surf})}{dt} = Q_{cut}(t) - K_{ds} \times Q_{surf}(t) \qquad \text{(Eq. 1)}$$

113
$$\frac{d(Q_{sc})}{dt} = K_{ds} \times Q_{surf}(t) - K_{dv} \times Q_{sc}(t) \qquad \text{(Eq. 2)}$$

114
$$\frac{d(Q_{Sk})}{dt} = K_{dv} \times Q_{sc}(t) + F_{Sk} \times \left(C_{art}(t) - \frac{C_{Sk}(t)}{PC_{Sk:Bl}} \right) \qquad \text{(Eq. 3)}$$

115   where Q_{surf} is the amount on the skin's surface, Q_{cut}, Q_{sc} and Q_{Sk} are respectively the amounts applied on

116   the skin, in the strateum corneum, and in the vascularized tissue (skin), F_{Sk} is the entering blood flow in

117   skin, C_{art} and C_{Sk} are respectively the concentrations in arterial blood and skin, $PC_{Sk:Bl}$ is the skin:blood

118   partition coefficient, and K_{ds} and K_{dv} (h^{-1}) are the uptake rates between the skin's surface and the stratum

119   corneum and between the stratum corneum and the skin compartment respectively.

120   Experimental studies in rodents showed that the distribution of pyrethroids is blood flow-limited in most

121   of the compartments except the ones with a physiological barrier or slowly perfused (*i.e.*, brain, fat and

122   muscle) (Mirfazaelian et al., 2006; Tornero-Velez et al., 2012; Willemin et al., 2016). These

123   compartments were then subdivided into a vascularized compartment and an intracellular space. The

124   exchanges between the two sub-compartments are governed by a permeability coefficient. In a

125   compartment T, the quantities in the vascular (Q_{blT}) and the intracellular spaces (Q_T) are respectively:

126
$$\frac{d(Q_{blT})}{dt} = F_T \times \left(C_{art}(t) - C_{blT}(t) \right) - PA_T \times \left(C_{blT}(t) - \frac{C_T(t)}{PC_{T:Bl}} \right) \qquad \text{(Eq. 4)}$$

127
$$\frac{d(Q_T)}{dt} = PA_T \times \left(C_{blT}(t) - \frac{C_{T(t)}}{PC_{T:Bl}} \right) \qquad \text{(Eq. 5)}$$

128   where F_T is the entering blood flow in the tissue, C_{art} the concentration in the arterial blood, C_{blT} the

129   concentration in the vascular space of the compartment, C_T the concentration in the tissue, PA_T (L/h) the

130   permeability coefficient, and $PC_{T:Bl}$ the tissue:blood partition coefficient.

131   Absorbed pyrethroids are eliminated from the body by metabolism. Metabolism in humans is rapid with

132   a half-life in blood of few hours; it occurs in the liver and the intestines (Crow et al., 2007; Godin et al.,

6

133 2007; Gotoh et al., 1998). Metabolism was described by a first order process. The total rate of

134 metabolism (R_{PYR}, mg/h) is therefore:

$$R_{PYR}(t) = R_{liv\_PYR}(t) + R_{GI\_PYR}(t) \qquad \text{(Eq. 6)}$$

$$\text{with } R_{T\_PYR}(t) = CL_{T\_PYR} \times C_{T\_PYR}(t) \qquad \text{(Eq. 7)}$$

137 where R_{T_PYR} (mg/h) is the metabolic rate of pyrethroids in the tissue T (here liver and GI tract), CL_{T_PYR}

138 (L/h) the clearance in the tissue T, and C_{T_PYR} (mg/L) the concentration in the tissue T.

2.3 Model structure for metabolites

Simple one-compartment models were used to describe the toxicokinetics of the metabolites, *i.e.* their

formation and urinary elimination. The formation of the metabolite *MET* from the pyrethroid *PYR*

($RoF_{PYRtoMET}$) was expressed as a fraction ($Frac_{PYRtoMET}$) of the global metabolic rate of *PYR* (R_{PYR}, Eq. 6)

(Willemin et al., 2016):

$$RoF_{PYRtoMET}(t) = Frac_{PYRtoMET} \times R_{PYR}(t) \times \frac{MW_{MET}}{MW_{PYR}} \qquad \text{(Eq. 8)}$$

145 where MW_X is the molecular weight of the chemical *X*. The total formation rate of the metabolite *MET*

146 was obtained by summing the formation rates from all its parent compounds. The resulting amount of

147 the metabolite *MET* (A_{MET}) was then obtained by subtracting the urinary elimination:

$$\frac{dA_{MET}}{dt} = \sum_{X \in PYR} RoF_{XtoMET}(t) - K_{uri\_MET} \times A_{MET}(t) \qquad \text{(Eq. 9)}$$

149 with K_{uri_MET} (h^{-1}) the urinary excretion rate constant. The concentration in urine was obtained by

150 dividing the amount of metabolite formed with the urinary flow (F_{urine}, L/h).

2.4 Model parameterization for parent compounds

The values of the PBPK model's parameters are reported in Table 2. The physiological parameters of

the PBPK models, *i.e.* bodyweight, and organ volumes and perfusion, were implemented as proposed by

Beaudouin et al. (2010) for men and women. The additional parameters (the blood volume fractions,

BV) were obtained from Brown et al. (1997) (BV$_{fat}$ = 0.02; BV$_{brain}$ = 0.03; BV$_{muscle}$ = 0.04).

156 The compound-specific parameters were set using published experimental or computational studies.

157 Because of the variety of the study designs (*e.g.* organisms (humans or rodents) and cells), a priority

158 order was defined to select the study that would be used for setting a parameter's value: *in vivo* human

159 *data, in vitro* human data, *in vivo* animal *data,* and QSAR predictions. Studies with separate isomers

160 experiments were also preferred to experiments done with a combination of isomers.

161 Oral absorption and excretion parameters were set using animal data for deltamethrin and *cis-* and *trans-*

162 permethrin (Godin et al., 2010; Willemin et al., 2016). The parameter estimates were obtained by

163 calibrating a PBPK model with animal toxicokinetic data. As no data were available for cypermethrin

164 and cyfluthrin, the values of permethrin (isomer specific) were used for these compounds. For dermal

165 absorption, the empirical values estimated by Tornero-Velez et al. (2012) for permethrin were used for

166 all the compounds.

167 Distribution in the tissues is governed by the tissue:blood partition coefficients and the permeability

168 coefficients for diffusion-limited compartments. Similarly to absorption, estimates obtained from

169 toxicokinetic experiments in rats were used for deltamethrin and permethrin (Godin et al., 2010;

170 Willemin et al., 2016). Since no *in vivo* data for cypermethrin and cyfluthrin were available, the isomer

171 specific estimates of permethrin were used for the permeability coefficients, and a QSAR model

172 developed by Knaak et al. (2012) or the tissue:blood partition coefficients.

173 Metabolic clearances (CL_{T_PYR} in Eq. 7) were defined with human *in vitro* studies using hepatocytes

174 (Willemin et al., 2015) or hepatic microsomes (Godin et al., 2006; Scollon et al., 2009). Because no

175 isomer-specific data were available for cypermethrin and cyfluthrin, the same value was used for both

176 isomers. Experiments with β-cyfluthrin were used, as no data were available for cyfluthrin and as β-

177 cyfluthrin is an enriched isomeric form of the two biologically active diastereoisomeric pairs of isomers

178 of cyfluthrin.

179 **2.5   Model parameterization for metabolites**

180 The parameters of the one-compartment model for the metabolites are the fractions formed from parent

181 compounds ($Frac_{PYRtoMET}$ in Eq. 8), the urinary elimination rates (K_{uri_MET} in Eq. 9), and the urinary flow

182 (F_{urine}). Toxicokinetic studies with controlled exposure of human volunteers were used to set values to

183 the compound-specific parameters (Leng et al., 1997b; Ratelle et al., 2015a, b; Sams and Jones, 2012;

184 Woollen et al., 1992). The metabolite fractions ($Frac_{PYRtoMET}$) were computed using the percentage of

185 the administered parent compound recovered as a metabolite corrected by the bioavailability (see

186 Supplementary Data for details). The urinary elimination rates (K_{uri_X}) were computed with the apparent

187 half-life ($t_{1/2}$) in urine obtained by the analysis of the elimination data provided in the experimental

188 studies:

189 $$K_{uri\_X} = \frac{\ln 2}{t_{1/2}} \qquad \text{(Eq. 10)}$$

190 When several values for $t_{1/2}$ were available, the average was used (see the Supplementary Data). The

191 parameters values for the metabolites' models are reported in Table 3. The urine flow rate (F_{urine}) was

192 set to 0.07 L/h for men and 0.05 L/h for women (ICRP, 2002).

2.6   Model evaluation

194 The global model was evaluated using all the human toxicokinetic studies. At least one study was

195 available for each parent compound: permethrin (Gotoh et al., 1998; Ratelle et al., 2015b; Tomalik-

196 Scharte et al., 2005), cypermethrin (Ratelle et al., 2015a; Woollen et al., 1992), cyfluthrin (Leng et al.,

197 1997a; Leng et al., 1997b), and deltamethrin (Sams and Jones, 2012). The characteristics of the *in vivo*

198 studies are presented in Table 4. The global model was applied and used to predict the measured data in

199 each of these studies. Monte Carlo simulations (10,000) were performed to simulate inter-individual

200 variability and uncertainty in parameters' values. Truncated normal distributions were assigned to the

201 compound-specific parameters (absorption, metabolism, partition coefficients, permeability coefficients)

202 with a variation of 30% of the mean value of the parameter and lower and upper bounds of 0.01% and

203 100% of the mean value of the parameter (except for fractions of metabolites that were truncated at 1).

204 Bodyweight was set to the values of study volunteers. For each simulation, a vector of random

205 parameter values was drawn from the probability distributions and was used as input of the global

206 toxicokinetic model.

2.7   Sensitivity analyses

207

208   Sensitivity analyses (SA) were conducted on the global model to identify the parameters with the most

209   impact on the blood concentrations of the parent compounds, and the urinary concentrations of the

210   metabolites. SA were also performed for the brain concentrations regarding the neurotoxicity of

211   pyrethroids under acute exposures. Continuous exposures similar for all parent compounds were

212   assumed: 1 ng/kg/day for oral intakes, 1×10^{-3} ng/L for air concentration, and 1 ng/day for dermal

213   contact. The sensitivity analyses were performed on concentrations at steady state.

214   We used two types of sensitivity analyses: first the Morris method to identify a subset of the most

215   influential parameters among the 199 parameters of the global model, then the Sobol's method on this

216   subset of parameters to quantify their impact on the concentrations of interest. The Morris method

217   (Morris, 1991) is a one-factor-at-a-time (OAT) method where the impact of changing the values of input

218   parameter is evaluated one by one in each run. It is a qualitative method providing a ranking of input

219   parameters in order of importance. The method is particularly well-suited when the number of

220   parameters is high. Each input factor may assume a discrete number of values, called levels (p), which

221   are chosen within the parameter range of variation. Three sensitivity measures are proposed for each

222   parameter: μ (the mean) and μ^* (the mean of the absolute values) that estimate the overall effect of the

223   factor on the output, and σ (the standard deviation) that estimates the ensemble of the second- and

224   higher-order effects in which the parameter is involved. Thus, a high value of μ^* means that the

225   parameter contributes to the dispersion of the output, and σ measures the linearity of the effects or the

226   interaction with the other parameters. The sample size is defined by a number, r. Uniform distributions

227   were assigned to the parameters of the global model with a coefficient of variation of 10% around their

228   mean values. The number of realizations (r) was set to 1,000 and number of levels (p) to 6.

229   Model parameters with the highest impact on the concentrations of interest were selected to run a global

230   sensitivity analysis with the Sobol's method (Saltelli et al., 2008; Sobol et al., 2007). This is a global

231   variance-based method, using the decomposition of the model output's variance into a sum of terms

232   depending on single factors (model parameters) and on interaction terms of higher order. It can handle

233   nonlinear and non-monotonic functions and models. Model output variances were estimated using

234   Monte Carlo integrals. Two independent input sample $n_1 \times p_1$ matrices (the "sample" matrix M1 and the

235   "resample" matrix M2), where n_1 (100,000) is the sample size and p_1 the number of parameters, were

236   used to compute the Monte Carlo integrals. Every row in M1 and M2 represents a possible parameter

237   combination. Two indices were computed: the first order index (FOI) that is the variance contribution of

238   one parameter to the total model variance, and the total order index (TOI) that is the result of the main

239   effect of the parameter and of its interactions with the other parameters.

240   **2.8   Application of the global model to the exposure of the French population**

241   The global model was used to link the external exposure of the four pyrethroids to the urinary

242   metabolite concentrations of the French population. The following exposure routes were considered: the

243   ingestion of contaminated food, the inhalation in indoor and outdoor environments, and the dermal

244   contact via suspended particles in air and sedimented dust.

245   2.8.1   Calculation of the aggregated exposure

246   Food ingestion was estimated using the pyrethroid concentrations measured different food groups

247   collected on the French market (ANSES, 2011) and the average consumption of food and liquids of the

248   French population (ANSES, 2009). The following equation was used to calculate the daily intake (D_{oral}

249   in mg/kg BW/day):

250    $$D_{oral} = \Sigma\ C_{food} \times IR_{food}$$ (Eq. 11)

251   with C_{food} the pyrethroids' concentration in a food group (mg/kg of food) and IR_{food} the intake rates for

252   adults (kg of food/kg BW/day) of the food group.

253   The method proposed by Hermant et al. (2017) for estimating the exposure from inhalation and dust of

254   permethrin was applied for all the pyrethroids of interest. Exposure from inhalation (C_{inh} in ng/m^3) was

255   estimated by combining exposure from indoor and outdoor contaminated air:

256    $$C_{inh} = (C_{in} \times T_{in}) + (C_{out} \times T_{out})$$ (Eq. 12)

257   where C_{in} and C_{out} (ng/m^3) are the indoor and outdoor concentrations respectively, and T_{in} (0.85) and T_{out}

258   (0.15) the fractions of the time spent indoors and outdoors respectively (Klepeis et al., 2001). The

259 indoor air concentrations were taken from Blanchard et al. (2014) and from The French Central

260 Laboratory of Air Quality Monitoring for outdoor air (LCSQA, 2009).

261 Dermal exposure is also possible by contact with suspended particles and sedimented dust (Weschler

262 and Nazaroff, 2008). The dermal contact with the airborne particles ($D_{dermal\_particles}$ in ng/d) was

263 calculated as follows (Shi and Zhao, 2014):

264 $$D_{dermal\_particles} = \left(vp \times C_{in} \times S \times Frac_{exp} \times T_{in}\right) + \left(vp \times C_{out} \times S \times Frac_{exp} \times T_{out}\right)$$ (Eq.

265 13)

266 where C_{in} and C_{out} (ng/m^3) are the indoor and outdoor concentrations respectively, T_{in} (h/d) and T_{out} (h/d)

267 are the length of time indoors and outdoors respectively, vp the deposition velocity of airborne particles

268 onto the skin's surface (m/h), $Frac_{exp}$ the fraction of body exposed (-), and S the body surface area (m^2).

269 The dermal contact with dust (D_{dermal_dust} in ng/d) was given by (Beko et al., 2013):

270 $$D_{dermal\_dust} = C_{dust} \times Frac_{exp} \times S \times Mp \times t$$ (Eq. 14)

271 where C_{dust} (ng/g) is the concentration in dust, $Frac_{exp}$ the fraction of body exposed (-), S the body

272 surface area (m^2), Mp the is the amount of dust adhering to skin (g/m^2) and t the daily exposure duration

273 (h/d). The aggregate dermal dose (D_{dermal} in ng/d) is obtained by summing these two exposure pathways:

274 $$D_{dermal} = D_{dermal\_dust} + D_{dermal\_particles}$$ (Eq. 15)

275 When a compound was not detected in food, outdoor or indoor air, or dust, the concentration was set to

276 the limit of detection divided by two when it was known (food and outdoor air), otherwise to the limit of

277 quantification divided by two (indoor air and dust) (EPA, 2000). None of the data used for the

278 calculation of the exposure scenarios makes a distinction between the isomers of a pyrethroid. The ratios

279 of *cis-* and *trans*-stereoisomers observed in the commercial formulations of the pyrethroids were then

280 used to compute the dose of each isomer. The *cis*:*trans* ratios usually observed in the commercial

281 formulations and used here are 40:60 for permethrin and cyfluthrin, and 42:58 for cypermethrin.

282   2.8.2   Study population and biomonitoring data

283   The French Nutrition & Health Survey (ENNS study) is a cross-sectional study carried out in the

284   general French population. It was performed between February 2006 and July 2007. The study

285   population included adults aged 18–74 years living in continental France in 2006–2007. About 400

286   adults (257 women and 139 men) participated in the pyrethroid pesticides study (Fréry et al., 2011).

287   Questionnaires were used to collect individual characteristics. For instance, the bodyweight of each

288   participant was recorded (65.49 ± 15.01 kg for women and 77.93 ± 12.69 kg for men). The Dubois and

289   Dubois (1989) equation was used to compute the body surface area with the weight and height of

290   participants (1.69 ± 0.18 m^2 for women and 1.92 ± 0.16 m^2 for men) that is used for the computation of

291   the exposure via dermal contact. One spot sample of first morning urines was also collected for each

292   participant in order to measure the major urinary metabolites of pyrethroids. The detection rates were

293   98.5% for 3-PBA, 83.1% for DBCA, 56.1% for *cis*-DCCA, 86.1% for *trans*-DCCA and 29.8% for F-

294   PBA (LOD = 0.03 µg/L and LOQ = 0.1 µg/L). The geometric mean of the quantified urinary metabolite

295   concentrations (µg/L) and the 95% interval of confidence were: 0.74 [3.41; 6.15] for 3-PBA, 0.37 [0.48;

296   4.20] for DBCA, 0.17 [0.67; 2.17] for *cis*-DCCA, 0.39 [2.44; 5.27] for *trans*-DCCA and 0.68 [0.64;

297   1.24] for F-PBA.

298   2.8.3   Exposure scenario and simulations

299   Monte Carlo simulations were performed to account for inter-individual variability and uncertainty.

300   Truncated normal distributions were assigned to the compound-specific parameters (absorption,

301   metabolism, partition coefficients, permeability coefficients, urinary excretion) with the mean value and

302   a coefficient of variation of 30%. The lower and upper bounds were defined as 0.01% and 100% of the

303   mean value (except for fractions of metabolites that are truncated at 1). The intakes were also affected

304   by uncertainty and variability. Normal distributions were assigned to all intakes (diet intakes, inhaled

305   concentration and applied dermal dose) with the mean value computed and a coefficient of variation of

306   500%. Normal distributions were also set to the bodyweight according to the actual distributions

307   observed in the ENNS study. For each Monte Carlo simulation (10,000), a vector of random parameter

308   values was drawn from the probability distribution functions and was used as input of the PBPK model.

309   A continuous scenario of exposure by inhalation, oral and dermal contact was considered, and the model

310   was run until steady-state in blood was achieved (1 month). The urinary concentrations of the five

311   metabolites were recorded after 1 month of exposure.

312   **2.9   Software**

313   GNU MC Sim v5.6.6 simulation software was used to implement the PBPK model (Bois, 2009). The R

314   package "sensitivity" was used (version 1.15.2) (Iooss et al., 2018): the "morris" method for the Morris

315   sensitivity analysis, and the "soboljansen" method for the Sobol sensitivity analysis.

316

3 Results

3.1 Model evaluation

We compared the experimental data observed in the human toxicokinetic studies with the corresponding model predictions (10,000 Monte Carlo simulations) (Figure 2). The data correspond to 189 measurements (concentrations or urinary excretion rates) related to five metabolites (3-PBA, F-PBA, *cis*-DCCA, *trans*-DCCA and DBCA) and one parent compound (permethrin). No human data were available to evaluate the toxicokinetics of the other three parent compounds predicted by the model. Figure 3 presents the comparison between the data and the model predictions, *i.e.* the average of the Monte Carlo simulations. Overall, the global model reproduced well the data: 98% of the predictions fall into the 10-fold interval with about 65% of the predictions in the 2-fold error interval, and 89% in the 3-fold one (Table S2 in Supplementary Data). The level of predictability is similar (about 60% in the 2-fold error interval) for the metabolites common to several pyrethroids (3-PBA, *cis*- and *trans*-DCCA) and is higher for the metabolites specific to one pyrethroid (DBCA and F-PBA), *i.e.* up to 90%. This could be explained by the fact that several studies were available for the common metabolites for model calibration and evaluation whereas only one study was available for DBCA and two studies, that were performed in the same laboratory, for F-PBA. The difference in predictability may then partly be a result of variability between studies. For permethrin (the only parent compound with data), only 9 data points were available for both isomers from one study (Gotoh et al., 1998). Most of the predictions were within the 3-fold error interval. It should be noted that, in this study, the exposure resulting from poisoning and was unknown. Moreover, only one person was involved whereas other studies involved several volunteers (except one study with cyfluthrin administered by ingestion).

Oral ingestion is the exposure route used in most of the studies (130 out of 189 data points). This route presents levels of predictability similar to the whole dataset, like the dermal contact route. For inhalation, predictability is better with almost all the predictions within the 2-fold interval (96%) but only one study for one parent compound (cyfluthrin) was available. The dermal route is the only route with predictions falling beyond the 10-fold error interval (about 11%), and they were all during the compound's absorption phase. We also compared the predictability between the absorption and

344 elimination phases (54 vs. 135 data points). Similar predictability levels were observed but all

345 predictions falling beyond the 10-fold interval were all during absorption.

346 In general, the toxicokinetic profiles are well reproduced by the model. For each profile, a confidence

347 interval calculated with the Monte-Carlo simulations was obtained. About 88% of the datapoints (165

348 over 189) are within the confidence intervals. The average profile is encompassed in the 95%

349 confidence interval with an upper bound corresponding to a 2-fold factor and a lower bound to a 0.2-

350 fold factor compared to the mean, but the width of the confidence interval tends to increase as time

351 since administration increases. The graphs of the toxicokinetic profiles with the 95% confidence interval

352 together and the experimental data are given in Figure S1 in the Supplementary Data for all studies.

353 Toxicokinetic parameters (maximum peak concentrations or excretion rates, time-to-peak levels and

354 elimination half-lives) were computed from the predicted toxicokinetic profiles (Table 5). The

355 maximum peak concentration or excretion rate and elimination half-live were well predicted by the

356 model, and in general the time to peak was slightly over-estimated. Our analysis of different data sets

357 also highlighted that the toxicokinetics of 3-PBA in urine observed in the study by Woollen et al. (1992)

358 differ from other studies (Ratelle et al., 2015a, b; Sams and Jones, 2012). The measured excretion rate

359 was very high and the elimination half-life quite low unlike the observations in the other studies and the

360 model predictions. Overall, all our results show that the global model is able to reproduce adequately the

361 urinary toxicokinetics of the metabolites with a high level of predictability.

362 **3.2   Sensitivity analyses**

363 The Morris method was used to select a subset of the 199 parameters that had the most influence on the

364 parent compounds concentrations in blood and brain, and on the urinary metabolite concentrations. The

365 Morris plots for all the model outputs of interest are provided in Figure S2 in the Supplementary Data.

366 We observed that each model output is sensitive to a small number of parameters, and that the low

367 standard deviation values indicate probably no interaction between parameters. We selected the 8 most

368 influential parameters towards urinary metabolite concentrations and the 4 most influential parameters

369 towards internal concentrations to run the variance-based Sobol's method. In the end, this selection

370 includes a total of 46 parameters (list given in Table S3 in the Supplementary Data). For all compounds,

16

371   these parameters are related to partitioning into the liver and brain, to metabolism and to absorption of

372   the parent compounds.

373   The first-order (FOI) and total order indices of the model parameters obtained by the Sobol analyses are

374   presented in Figure 3 for metabolites and Figure S3 in the Supplementary Data for the parent

375   compounds. For all outputs, the Sobol results confirmed the Morris analyses on the absence of

376   interaction between the model parameters meaning that the effects of the parameter on the model

377   outputs are independent. A relatively high uncertainty of the prediction of the FOI was observed that

378   may be a result of a high number of non-sensitive parameters integrated into the analysis and/or of the

379   correlations between the parameters. The parameters with a high influence on blood and brain

380   concentrations were absorption and excretion parameters, partition coefficient between liver and blood,

381   liver clearance and diffusion in brain. These results are in agreement with previous published sensitivity

382   analyses on the toxicokinetics of permethrin and deltamethrin (Darney et al., 2018; Godin et al., 2010;

383   Mirfazaelian et al., 2006; Wei et al., 2013).

384   For the urinary metabolites' concentrations, the results showed that urinary metabolite concentrations

385   are mostly influenced by the fractions of the metabolic rates of parent compound that lead to each

386   metabolite ($Frac_{PYRtoMET}$). Some parameters related to absorption (*i.e.* absorption from stomach, gut and

387   stomach-gut transfer) also influenced urinary metabolite concentrations to a lesser extent. Furthermore,

388   the sensitivity analyses allowed to classify the parent compounds in terms of influence on the urinary

389   concentrations of the common metabolites. For *cis-* and *trans*-DCCA, the ranking is: permethrin,

390   cypermethrin and cyfluthrin. For 3-PBA, we obtained: *trans*-permethrin, *trans*-cypermethrin, *cis*-

391   permethrin and, to a lesser extent, *cis*-cypermethrin and deltamethrin. Finally, regarding F-PBA which

392   is specific to cyfluthrin, the *trans* isomer appears to have a higher influence than the *cis* isomer.

393   **3.3   Simulation of urinary levels in the French population**

394   The exposure doses calculated for the French population for the three exposure routes are presented in

395   Table 6. A large part of the calculated oral daily intakes comes from the fact that in samples where no

396   pyrethroids were detected, the LOQ or LOD divided by 2 was used. That tends to smooth the daily

397   intakes between the pyrethroids. Indeed the detection rates in food were very low (<1%) for pyrethroids

398   in all the food items and none of the investigated pyrethroids were detected in tap water (ANSES,

399   2011). That could be due to the rather high limits of detection (LOD) (5 µg/kg for cyfluthrin and

400   cypermethrin and 3 µg/kg for permethrin and deltamethrin). Regarding the other routes, the frequency

401   of quantification in dust was 84% for permethrin (n=25, LOQ = 0.09 µg/g) and 52% for cypermethrin

402   (n=23, LOQ = 0.06 µg/g) (Blanchard et al., 2014). For airborne particles, the frequencies of

403   quantification were 40% for permethrin (n = 30, LOQ = 0.002 ng/m^3) and 3% for cypermethrin (n=30,

404   LOQ = 0.2 ng/m^3) (Blanchard et al., 2014). For outdoor air, the samples came from 12 French regions

405   and were taken between 2001 and 2007. The sample sizes ranged from 42 for permethrin to 2,428 for

406   cypermethrin, and the frequencies of quantification ranged from 0% (permethrin and cyfluthrin) to 0.3%

407   (deltamethrin) (LOQ = 0.071 ng/m^3) (LCSQA, 2009). According to our exposure estimates, the

408   population is exposed mainly to permethrin and cypermethrin and to a lesser extent to cyfluthrin and

409   deltamethrin for all the routes. Due to the use of commercial isomer formulations, the population is

410   more exposed to the *trans* isomer than to the *cis* one.

411   The predictions of the urinary metabolite concentrations by the global PBPK model using the calculated

412   exposures are consistent with the biomonitoring data of the ENNS study (Figure 4). The results were

413   similar between men and women. Because of the low quantification rates of *cis*-DCCA and F-PBA in

414   the study population (56.1% and 29.8% respectively), the predicted and measured distributions were

415   represented either without accounting for the non-quantified samples, or by assigning the LOQ value

416   (0.1 µg/L) to these samples and setting the model predictions below the LOQ to the LOQ. For all

417   compounds, the predicted median is always higher than the measured one (except for F-PBA when the

418   non-quantified samples are not considered). For men, the over-prediction factor is 3.5 for *trans*-DCCA,

419   3.3 for *cis*-DCCA, 1.4 for DBCA, 1.9 for 3-PBA, and 1.3 for F-PBA when the non-quantified samples

420   are considered. When they are not, these factors are respectively 2.8, 1.4, 1.2, 1.8, and 0.4. Our results

421   showed that the predictions are close to the measurements for metabolites specific to one compound, *i.e.*

422   DBCA (deltamethrin) and F-PBA (cyfluthrin). For 3-PBA, the difference is less than two-fold but not

423   all the pyrethroids parents forming that metabolite are taken into account in our analysis. Indeed, we

424   considered three parent compounds (permethrin, cypermethrin and deltamethrin) among the eight parent

compounds that can form 3-PBA. For *cis* and *trans*-DCCA, all the parent compounds are considered and the difference between predictions and measurements are slightly higher than the 3-factor (median). The predicted distributions cover a large range of values that is a result of the inter-individual variability and the uncertainty in the exposure doses and in the PBPK parameters related to the physiology and biochemistry. However, we observed that the maximal values were always under-predicted by the model by a factor ranging from 0.7 for 3-PBA (pred: 5.2 µg/L; obs: 7.8 µg/L) to 0.1 for F-PBA (pred: 1.0 µg/L; obs: 12.3 µg/L) in men. In women, the under-prediction factor ranged from 0.4 for F-PBA (pred: 1.8 µg/L; obs: 4.5 µg/L) to 0.1 for *trans*-DCCA (pred: 5.5 µg/L; obs: 60.7 µg/L). We also observed that, unlike the predicted maximal value, the measured maximal value is highly variable between men and women.

According to our assumptions on the exposure doses, we assessed the contributions of the three exposure routes to the metabolite concentrations in urine. The oral exposure is the major route as the inhalation and dermal exposures account for less than 1% of urinary concentrations. The contributions of the different pyrethroids to the urinary concentrations are presented in Figure 5 for men (similar results for women, not shown). These results are the average of the contributions calculated with the 10,000 Monte Carlo simulations. The cumulative concentrations of *cis*- and *trans*-DCCA were formed by 45% of permethrin, 33% of cypermethrin, and 22% of cyfluthrin. These contributions are quite similar to the contributions to the global intake of parent compounds that could form *cis*- or *trans*-DCCA. Indeed, permethrin represents 42% of the global intake, cypermethrin 33%, and cyfluthrin 25%. The situation is different for 3-PBA, a metabolite common to permethrin, cypermethrin and deltamethrin. The contributions to the urinary concentrations were 61% for permethrin, 26% for cypermethrin and 13% for deltamethrin whereas the contributions to the global intake were, respectively, 40%, 30% and 30%. Deltamethrin contributes more to the global intake than to the 3-PBA concentrations, whereas it is the opposite for permethrin. F-PBA is generated from the two isomers of cyfluthrin whose contributions were 72% for the *trans* isomer and 28% for the *cis* isomer. The *trans* isomer has an increased contribution to the urinary concentrations compared to the intake (60% vs. 40%).

4   Discussion

The aim of our work was to propose a global toxicokinetic human model for four widely used pyrethroids (deltamethrin, permethrin, cypermethrin, and cyfluthrin including their *cis* and *trans* isomers) and their major urinary metabolites. This global model accounts for multi-route exposures (ingestion, inhalation and dermal contact) and for cumulative exposure to pyrethroids that share common metabolites.

4.1   Construction and evaluation of the global PBPK model

The PBPK models for the parent compounds were parameterized with numerous studies employing various methods (*in vitro*, *in vivo* and *in silico*). Previous studies in rats (Tornero-Velez et al., 2012; Willemin et al., 2016) showed that the permethrin isomers (*cis* and *trans*) exhibit slightly different toxicokinetic profiles, with a rapid absorption and a longer residence time for the *cis*-permethrin compared to the *trans* isomer. We therefore chose to build specific PBPK models for the *cis* and *trans* isomers of permethrin, cypermethrin and cyfluthrin. Among the four pyrethroids parent compounds studied, the toxicokinetics of permethrin and deltamethrin were well characterized as they have been studied in animals and then extrapolated to humans (Darney et al., 2018; Godin et al., 2010; Mirfazaelian et al., 2006; Tornero-Velez et al., 2012; Willemin et al., 2016). On the contrary, the toxicokinetics of cyfluthrin and cypermethrin have not been studied *in vivo*. Their PBPK model parameters were therefore informed using *in vitro* or *in silico* studies or set to the values of the other two pyrethroids, notably for the absorption or permeability in organs. Because there were no available data for cyfluthrin and cypermethrin, we assumed that their isomers behave similarly in terms of partitioning into the tissues (predicted by QSAR) (Knaak et al., 2012) and hepatic clearances (*in vitro* experiments) (Scollon et al., 2009). The performance of the QSAR model was checked by comparing its predictions to the *in vivo* data for permethrin and deltamethrin. A factor of about 3 was observed between the experimental and calculated values (except for brain for which the difference was much higher), that is an acceptable error for QSAR predictions. Since there were no data for absorption and permeability of cyfluthrin and cypermethrin, data related to the specific isomers *cis*- and *trans*-permethrin were used for these processes. Globally, the values of the model's parameters are of the same order for all the parent

479   compounds. For instance, the hepatic clearances are relatively close, between 1.56 L/h/kg for *cis-*

480   permethrin and 9.72 L/h/kg for deltamethrin, as well as the partitioning into the tissues. As a result, the

481   toxicokinetics of the parent compounds exhibit similar profiles. Even if the pyrethroids of interest are

482   metabolized by the same enzymes (cytochromes P450 and carboxylesterases), we did not model

483   metabolic interactions in the global model as we used it for environmental low doses. Moreover, as

484   observed *in vitro* in human hepatocytes for permethrin (Willemin et al., 2015), the interaction between

485   the *cis* and *trans* isomers was assumed negligible.

486   Unlike the parent compounds, the one-compartment models for metabolites were exclusively

487   parametrized with *in vivo* data, most of them being observed in controlled human volunteer exposures

488   (Leng et al., 1997a; Leng et al., 1997b; Ratelle et al., 2015a, b; Sams and Jones, 2012; Woollen et al.,

489   1992). In most of these studies, the parent compound was administered as a mixture of isomers and the

490   production of metabolites was reported as the percentage of the administered dose. For common

491   metabolites of isomers (3-PBA, and F-PBA), it was therefore not possible to distinguish the proportion

492   of the metabolite produced by each of the isomers. In a way similar to what Tornero-Velez et al. (2012)

493   did, we used the study of Gaughan et al. (1977) to estimate these proportions. From that study, the parts

494   of 3-PBA produced by the *cis* and *trans* isomers of permethrin were calculated, as the animals were

495   exposed to each isomer separately. The same ratios (*cis/trans*) were then applied to the other parent

496   compounds after correcting by the administered isomeric ratio. This method was applied to permethrin

497   (3-PBA), cypermethrin (3-PBA) and cyfluthrin (F-PBA). Globally, the parameters' values of the one-

498   compartment models for metabolites are in the same range. It can be noticed that the metabolite fraction

499   of the *trans* isomers are always higher than the *cis* ones. Regarding the results of the sensitivity

500   analyses, the fraction of the parent compound that is transformed into a metabolite greatly affects the

501   model predictions of urinary metabolites' concentrations. The uncertainties lying in the extrapolation of

502   the isomer ratios to common metabolites from one compound to another could be reduced by collecting

503   new experimental data, and this will therefore improve the quality of the model predictions.

504   We chose to use most of all the human data available on the toxicokinetics of the pyrethroids and

505   metabolites of interest. This enables to derive parameter's values, especially for the metabolic fractions,

506 accounting for the variability between the different studies. Only one incoherence between studies was

507 revealed, which was the toxicokinetic of 3-PBA in urine observed in the study by Woollen et al. (1992)

508 that differs from the other studies following that metabolite (Ratelle et al., 2015a, b; Sams and Jones,

509 2012). Nevertheless, some information is still missing, and assumptions had to be made. The model

510 could be easily updated to include any new data that could refine quantitatively some processes or

511 isomer-specific data, especially for cyfluthrin and cypermethrin. Our methodology for the

512 parameterization of the compound-specific parameters has provided good predictability of the global

513 model. Most of the toxicokinetic data (studies other than the one used for parameterization) were

514 reasonably simulated given uncertainties of model parametrization data and data used to evaluate the

515 model, i.e. 65% and 89% of the model predictions fall into the 2-fold and 3-fold error interval

516 respectively. The development of our model fulfills the key principles and best practices for

517 characterizing and applying PBPK models in risk assessment defined by the World Health Organization

518 (IPCS, 2010), in terms of model documentation, evaluation, and statistical analyses performed.

519 **4.2   Prediction of the urinary concentrations in the French adult population**

520 We tested the global model to predict the urinary concentrations of the five pyrethroids' metabolites

521 based on the estimation of the cumulative and aggregate exposure of the French adult population to the

522 four pyrethroids of interest. This application aimed at demonstrating that the global model together with

523 an adequate scenario of exposure was able to reproduce biomarkers of exposure commonly measured in

524 biomonitoring studies, here the ENNS study (Fréry et al., 2011). To derive realistic exposure doses, we

525 followed the methodology developed for permethrin by Hermant et al. (2017) for inhalation, dermal

526 contact and dust, and by Darney et al. (2018) for food, but selected different datasets for the food and

527 outdoor air contamination that are more representative of the French national exposure to be in

528 concordance with the ENNS biomonitoring data. We then selected the outdoor air measured in 12

529 French regions between 2001 to 2007 (LCSQA, 2009). For food contamination, Darney et al. (2018)

530 used data obtained by the French Ministry in charge of consumption (DGCCRF, 2008), the French

531 Ministry in charge of agriculture (DGAL) and the French Ministry in charge of Health (DGS). These

532 analyzes have been carried out within the framework of targeted controls for nonconformities observed

533   previously or during reinforced controls on imports. In this work, we chose to use Total Diet Studies 2

534   that aimed to measure numerous chemicals in different food samples taken from the French market

535   (ANSES, 2011). Besides the sampling scheme, the main difference between the two studies was the

536   levels of detection of pyrethroids in the food samples that was a bit higher for the first studies but still

537   quite low (not superior to 3% in all media). The low detection rate of the Total Diet Studies 2, that we

538   used, was a consequence of the choice of the analytical method that had to be suitable for a large

539   number of chemicals and was therefore not very sensitive to pyrethroids, with LOD between 3 µg/kg

540   and 100 µg/kg. As a comparison, Melnyk et al. (2014) obtained LOD between 0.05 µg/kg and 0.8 µg/kg

541   in a similar study on pesticides only. This low detection had an impact on our diet intake estimates as

542   we chose to replace the non-detected concentrations by the LOD divided by 2. This also explains why

543   the computed diet intakes are rather similar between the pyrethroids of interest, with a maximum of

544   0.020 µg/kg/d for permethrin and minimum of 0.012 µg/kg/d for cyfluthrin. In the end, the choice of the

545   studies on food and air contamination has a little impact on the calculated exposure doses as the ones we

546   obtained for permethrin are quite similar to the intermediate exposures in France calculated in the

547   previous works (Darney et al., 2018; Hermant et al., 2017). Despite the uncertainties in the French food

548   contamination data, our exposure estimates are close to exposures calculated in other countries, the

549   United States (Melnyk et al., 2014), Hong Kong (Wong et al., 2014) and Spain (Quijano et al., 2016).

550   For instance, Melnyk et al. (2014) estimated diet intakes slightly lower than our estimates, *i.e.* 0.018 vs.

551   0.02 µg/kg/d for permethrin, 0.0095 vs. 0.015 µg/kg/d for cypermethrin, and 0.003 vs. 0.012 µg/kg/d for

552   cyfluthrin. All our diet estimates are also well below the admissible daily intakes from World Health

553   Organization are between 3 and 250 µg/kg/day (respectively cyfluthrin and permethrin) for most

554   common used pyrethroids (IPCS, 2009).

555   The aim of this part of the work was to provide reasonable exposure estimates to test the behavior of our

556   global model. The calculated exposures were then used as inputs of the global model to predict the

557   urinary concentrations of the five metabolites. Our results show that the model's predictions are in good

558   agreement with the measured concentrations in the ENNS study (Fréry et al., 2011). At maximum, a

559   factor of 3 was observed between the medians of the predicted and observed distributions. The maximal

560 observed concentrations are not well reproduced. The predictions could be improved by refining and

561 individualizing the exposure scenarios. Indeed, pyrethroids are non-persistent compounds whose urinary

562 concentrations are expected to greatly vary over the day according to the contact with the chemical and

563 the time of sampling (Aylward et al., 2017). Because no such data was available, we assumed a constant

564 exposure throughout the day, which probably does not reflect real-life (diet) exposure. Other

565 information such as diet, presence of animals at home, proximity to growing fields or the use of

566 insecticides at home could be useful (Dereumeaux et al., 2018).

567 **4.3   Application in biomonitoring studies**

568 One of the utilities of our global model will be to facilitate the interpretation of the biomarkers of

569 exposure collected in biomonitoring studies for pyrethroids. Such a model will be an adequate tool to

570 study the toxicokinetic of several pyrethroids and metabolites at the same time. That is an improvement

571 of the current practices that consist in dealing with each compound separately (Cote et al., 2014; Darney

572 et al., 2018; Tornero-Velez et al., 2012; Wei et al., 2013). We performed several analyses to test the

573 model behavior in that specific context. For instance, we identified the model parameters that influence

574 the most the urinary concentrations under a constant exposure. It turned out that the toxicokinetics of the

575 parent compounds have a little impact for this exposure scenario, and that the urinary concentrations

576 were mainly driven by the metabolic fractions, that represent the fraction of the parent metabolism that

577 is transformed in a specific metabolite. These results depend not only on the toxicokinetics but also on

578 the chosen exposure scenario (steady-state at constant exposure). Under a real-life exposure scenario

579 (several short exposures over the day), some other parameters, like the urinary excretion, will probably

580 impact the urinary metabolites' concentrations. Another interesting result of the sensitivity analyses run

581 with the global model was the ranking of the compounds in terms of impact on the urinary

582 concentrations. For instance, the ranking order was: permethrin > cypermethrin > cyfluthrin for DCCA

583 and permethrin > cypermethrin > deltamethrin for 3-PBA. As we assumed that the proportion of the

584 *trans* isomer was always higher of the *cis* one in the administered doses, that ranking is also found in the

585 sensitivity analyses results. Similarly, the ranking of the exposure routes was possible. As already

586 observed with the ENNS study, our results highlighted that the oral exposure was the major route of

587 exposure and inhalation and dermal exposure seems to be minor pathways. Overall, these results

588 highlight the most important parameters of the model for the analysis of the metabolite urinary

589 concentrations. The refinement of the input data for the most sensitive parameters (through additional

590 experimental data) can support an improvement of the model prediction capabilities.

591 **4.4   Conclusions**

592 In conclusion, we developed a global model for pyrethroids in humans using *in vivo*, *in vitro* and in

593 *silico* data. This model will be a useful tool to interpret biomonitoring data for pyrethroids that are

594 urinary concentrations of their metabolites that can be common to several pyrethroids (*e.g.*, 3-PBA and

595 DCCA). The global model combined with realistic cumulative and aggregated exposures to permethrin,

596 cypermethrin, cyfluthrin and deltamethrin was tested for the French population. The results and the

597 possible related analyses are promising for the application of this model in the context of biomonitoring

598 studies and more generally in risk assessment. While this study focused on adults, the model could be

599 easily adapted to children that are a more sensitive population in terms of exposure (Barr et al., 2010;

600 Egeghy et al., 2011; Schulz et al., 2009) and effects (Eskenazi et al., 2018; Farag et al., 2007; Shafer et

601 al., 2005). The PBPK model already integrates the physiological and anatomical changes due to age.

602 Recently, several works studied the *in vitro* metabolism and the toxicokinetics of few pyrethroids in

603 immature animals (Amaraneni et al., 2017; Kim et al., 2010; Mortuza et al., 2018; Nallani et al., 2018;

604 Song et al., 2019).

7   References

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Figure 1. The global PBPK model for a pyrethroid and the urinary metabolites F-PBA, DBCA, 3-PBA and *cis*- and *trans*-DCCA.

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841 **Figure 1. The global PBPK model for a pyrethroid and the urinary metabolites F-PBA, DBCA, 3-**
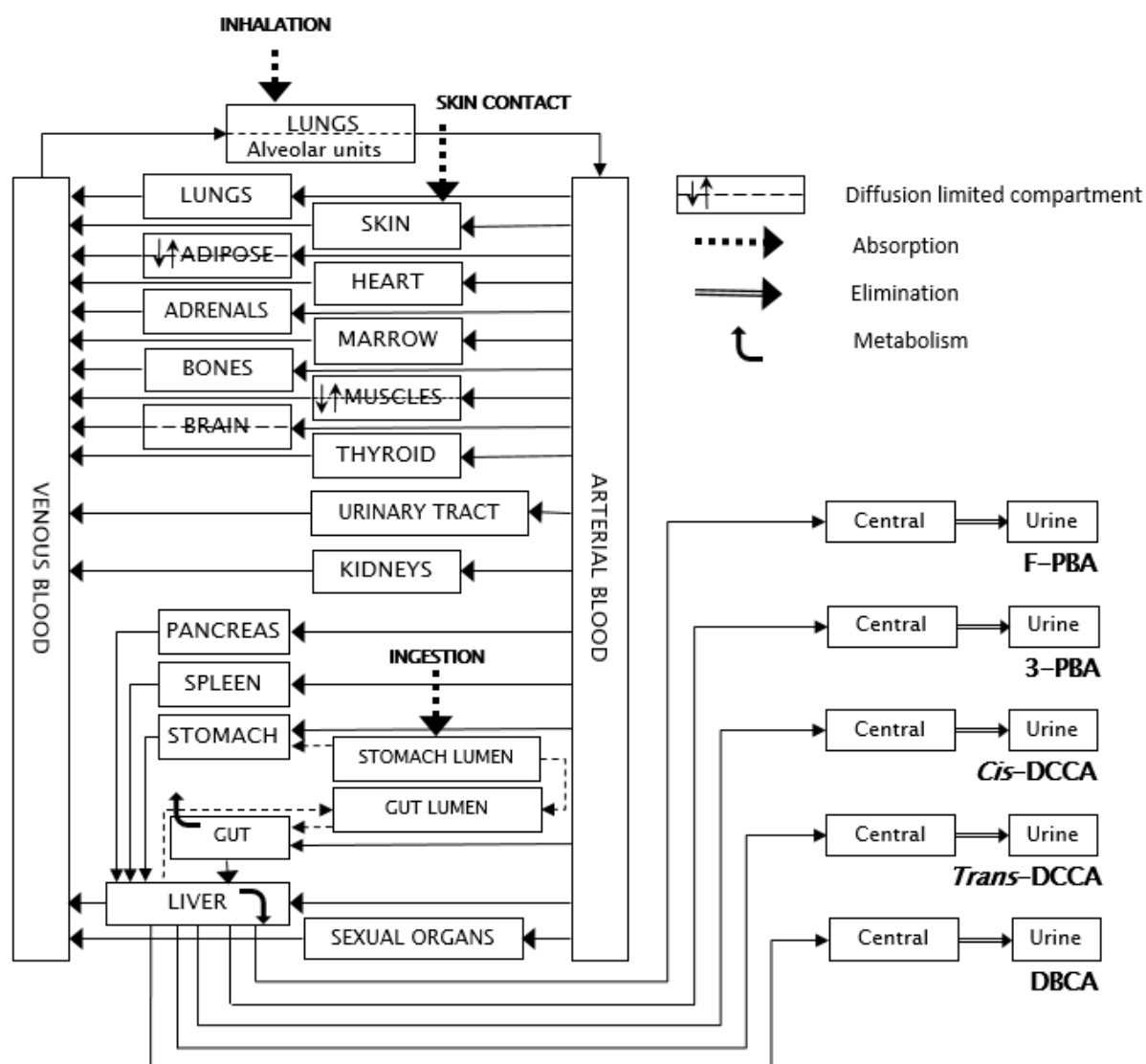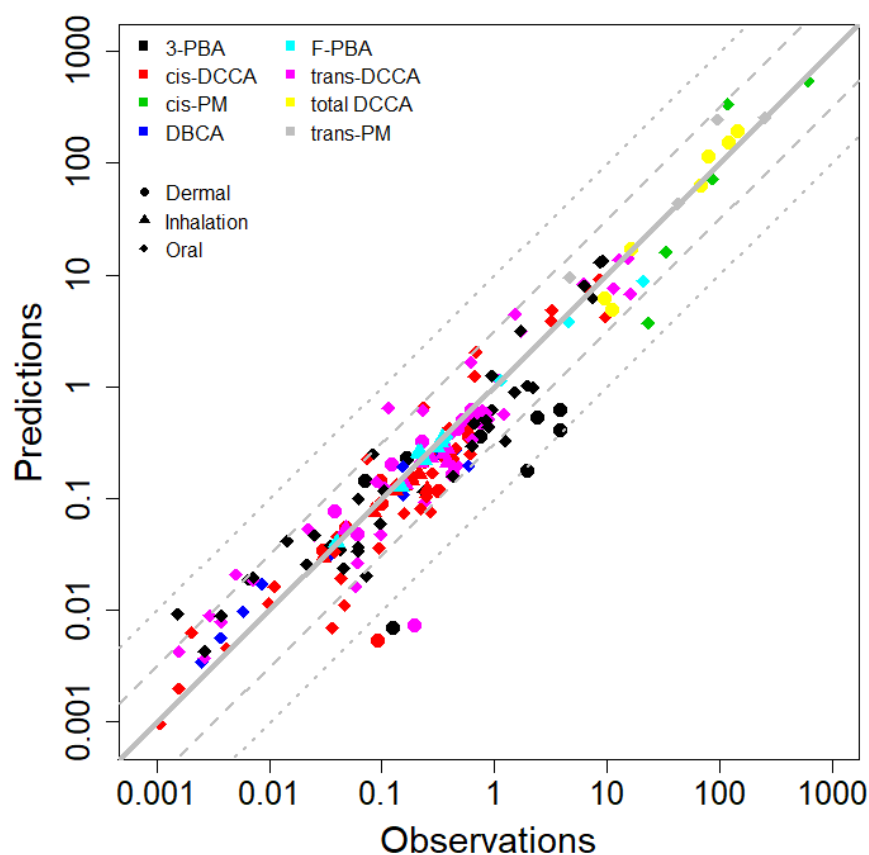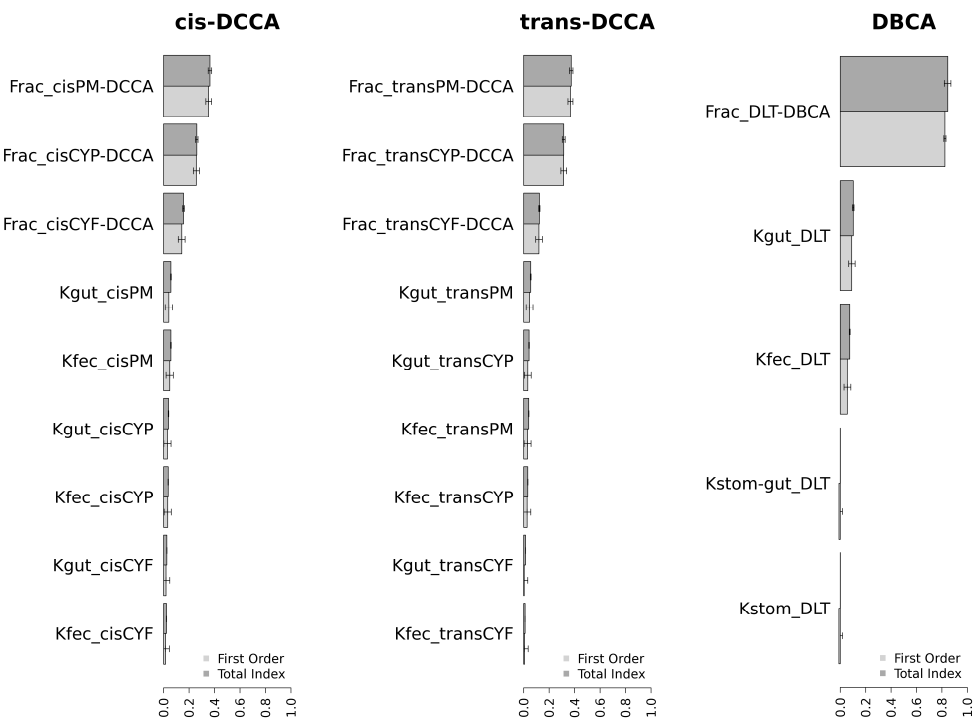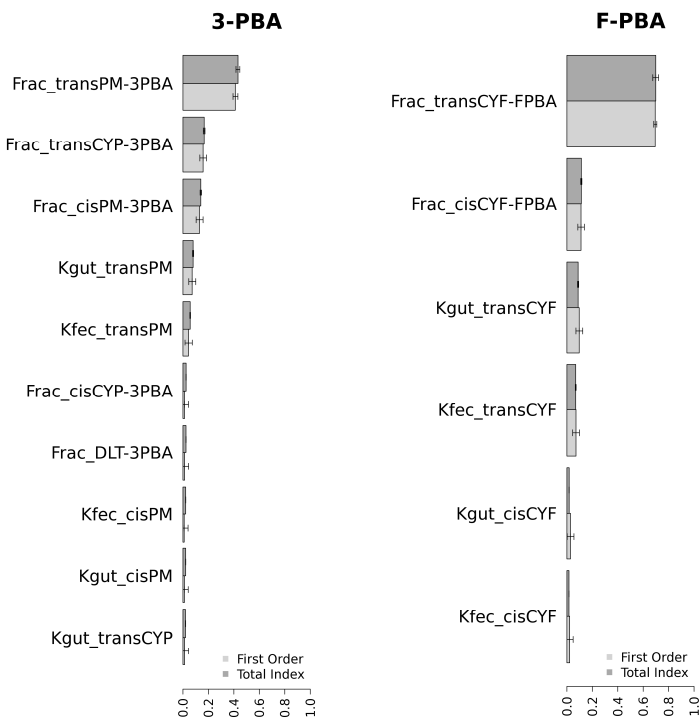842 **PBA and *cis*- and *trans*-DCCA.**

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Figure 2. Comparison of the model predictions with experimental data obtained in humans after oral, dermal or inhalation exposure to one pyrethroid (Gotoh et al., 1998; Leng et al., 1997a; Leng et al., 1997b; Ratelle et al., 2015a, b; Sams and Jones, 2012; Tomalik-Scharte et al., 2005; Woollen et al., 1992). The five metabolites are represented (3-PBA, F-PBA, *cis*-DCCA, *trans*-DCCA, DBCA) and *trans*- and *cis*-permethrin. The plain line is the perfect correspondence between the predictions and the experimental data. The dashed lines represent the 5-fold error interval, and the dotted lines the 10-fold error interval.

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Figure 3. First-order (light grey) and Total (dark grey) Sobol sensitivity indices for urinary concentrations of cis-DCCA, trans-DCCA, 3-PBA, F-PBA and DBCA following exposure to cis- and trans-permethrin, cis- and trans-cypermethrin, cis- and trans-cyfluthrin and deltamethrin.
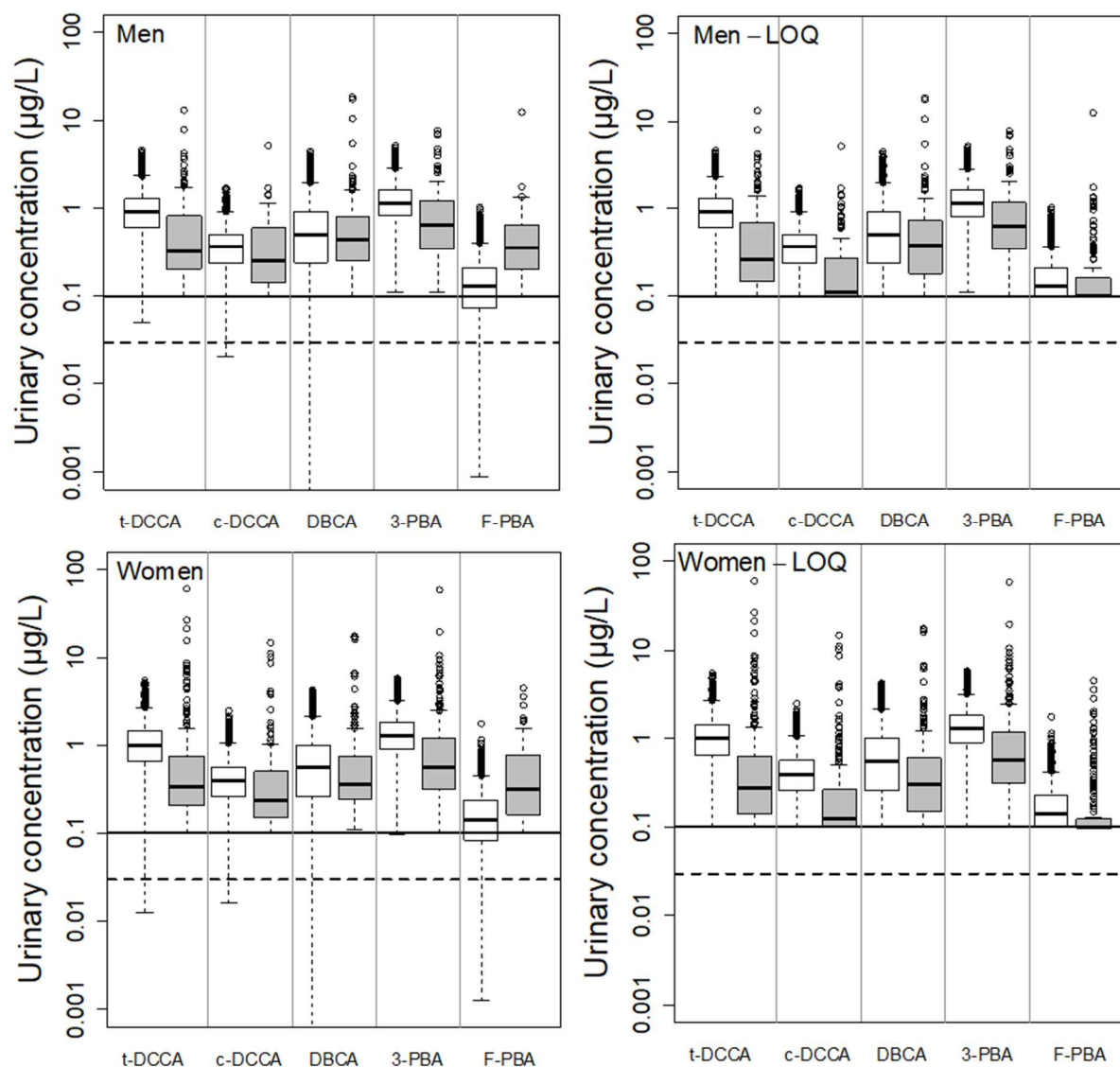
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Figure 4 Predictions of urinary concentrations after cumulative exposures to pyrethroids:
comparison of simulated (white boxplot) and measured (grey boxplot) of urinary concentration of
***cis*-DCCA, *trans*-DCCA, 3-PBA, F-PBA and DBCA for men and women. The black plain line is**
the limit of quantification (0.1 μg/L), and the dotted line the limit of detection (0.03 μg/L). The
percentage of quantification of urinary metabolites were 98.5% for 3-PBA, 56.1% for *cis*-DCCA,
86.1% for *trans*-DCCA, 29.8% for F-PBA and 83.1% for DBCA. The left panels present the
distributions of the measured metabolite concentrations without the non-quantified samples,
whereas in the right panels the predicted and measured distributions were truncated to the LOQ,
i.e. the LOQ was assigned to the measured and predicted concentrations that were below the
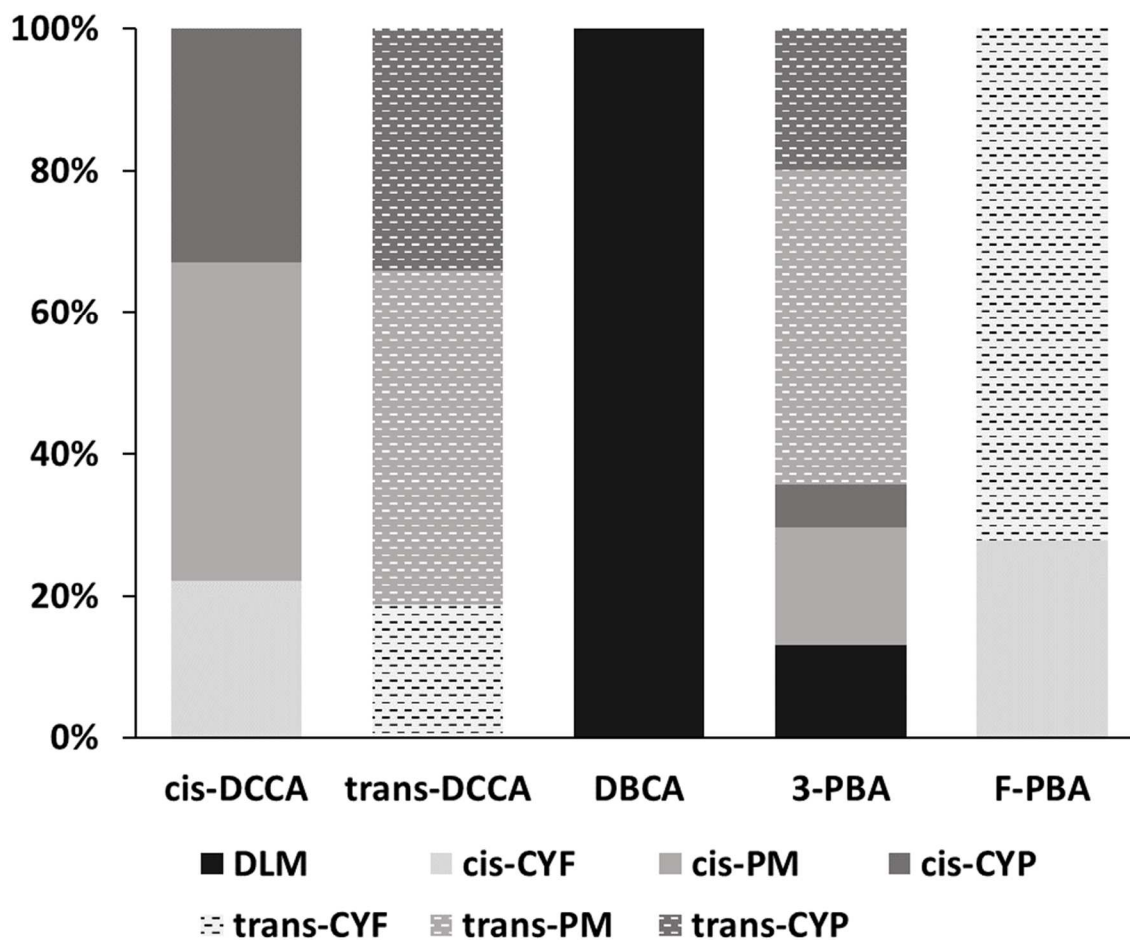**LOQ.**

Figure 5. Contributions of parent compounds to the urinary metabolite concentrations predicted by the global model following the oral, dermal and inhalation exposures calculated for the French population. The results are the average of 10,000 Monte Carlo simulations. DLM stands for deltamethrin, CYF for cyfluthrin, CYP for cypermethrin, and PM for permethrin.

9   List of tables

892

893   **Table 1. Pyrethroid compounds and their urinary metabolites.**

Parent compounds	Metabolites
cis-permethrin	*cis*-DCCA, 3-PBA
trans-permethrin	*trans*-DCCA, 3-PBA
cis-cypermethrin	*cis*-DCCA, 3-PBA
trans-cypermethrin	*trans*-DCCA, 3-PBA
cis-cyfluthrin	*cis*-DCCA, F-PBA
trans-cyfluthrin	*trans*-DCCA, F-PBA
deltamethrin	DBCA, 3-PBA

894   DCCA: 3-(2,2-dichlorovinyl); 3-PBA: 3-phenoxybenzoic acid; F-PBA: 4-fluoro-3-phenoxybenzoic acid; DBCA: cis-3-(2,2-dibromovinyl)-2,2-

895   dimethylcyclopropane carboxylic acid

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Table 2. Parameter values of the PBPK model for the seven pyrethroids and isomers.

Parameters	cis-CYF	trans-CYF	cis-CYP	trans-CYP	cis-PM	trans-PM	DLT
Absorption and excretion rates (h^{-1})							
Absorption from stomach	0.01[c]	0.01[c]	0.01[c]	0.01[c]	0.01[c]	0.01[c]	0.01[c]
Absorption from Gut	0.52[b]	1.30[b]	0.52[b]	1.30[b]	0.52[b]	1.30[b]	1.51[g]
Stomach-gut transfer	0.35[b]	0.20[b]	0.35[b]	0.20[b]	0.35[b]	0.20[b]	0.42[g]
Fecal excretion rate	0.39[b]	0.85[b]	0.39[b]	0.85[b]	0.39[b]	0.85[b]	0.59[g]
Surface-SC transfer[e]	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
SC-venous blood transfer[e]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Tissue:Blood partition coefficients							
Adipose	31.31[a]	31.31[a]	31.11[a]	31.11[a]	225.00[b]	76.00[b]	75.00[g]
Adrenal	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Bone	2.17[a]	2.17[a]	8.68[a]	8.68[a]	1.10[b]	0.21[b]	8.10[g]
Brain	8.94[a]	8.94[a]	7.94[a]	7.94[a]	1.60[b]	0.57[b]	0.14[g]
Breast	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Heart	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Marrow	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Muscle	3.42[a]	3.42[a]	3.03[a]	3.03[a]	1.20[b]	0.82[b]	5.64[g]
Sexual organs	2.17[a]	2.17[a]	1.92[a]	1.92[a]	0.63[b]	0.21[b]	8.10[g]
Pancreas	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Skin	4.18[a]	4.18[a]	3.71[a]	3.71[a]	19.00[b]	8.40[b]	8.10[g]
Spleen	2.17[a]	2.17[a]	3.04[a]	3.04[a]	1.10[b]	0.21[b]	8.10[g]
Thyroid	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Urinary tract	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Kidney	3.36[a]	3.36[a]	2.99[a]	2.99[a]	1.10[b]	0.21[b]	8.10[g]
Lung	2.17[a]	2.17[a]	0.67[a]	0.67[a]	1.10[b]	0.21[b]	8.10[g]
Gut	2.17[a]	2.17[a]	6.26[a]	6.26[a]	1.10[b]	0.21[b]	8.10[g]
Stomach	2.17[a]	2.17[a]	1.92[a]	1.92[a]	1.10[b]	0.21[b]	8.10[g]
Liver	5.57[a]	5.57[a]	4.95[a]	4.95[a]	0.89[b]	0.89[*b]	19.0[g]
Permeability coefficients (L/h)							
Adipose	0.048[b]	0.11[b]	0.048[b]	0.11[b]	0.048[b]	0.11[b]	0.025[g]
Brain	0.001[b]	0.0012[b]	0.001[b]	0.0012[b]	0.001[b]	0.0012[b]	0.002[g]
Muscle	0.32[b]	0.48[b]	0.32[b]	0.48[b]	0.32[b]	0.48[b]	0.043[g]
Metabolic clearance (L/h/kg BDW)							
Intestinal	-	-	-	-	0.00[e]	0.78[e]	-
Liver	5.34[f]	5.34[f]	6.24[f]	6.24[f]	1.56[d]	3.97[d]	9.72[h]

[a]Knaak *et al.* (2012) ; [b]Willemin *et al.* (2016) ; [c]Mirfazaelian *et al.* (2006) ; [d]Willemin *et al.* (2015) ;

[e]Tornero-Velez *et al.* (2012) [f]Scollon et al. (2009) [g]Godin et al. (2010); [h]Godin et al. (2006); [*]equal to

the *cis* isomer (not determined *in vivo*)

Table 3. Parameter values for the metabolite one-compartment models. The references for the urinary excretion rates are given in Supplementary Data.

Parameters	Cis-DCCA	Trans-DCCA	DBCA	3-PBA	F-PBA
Metabolite fractions ($Frac_{PYRtoMET}$)					
trans-Permethrin	-	0.61[a]	-	0.85[a]	-
cis-Permethrin	0.37[a]	-	-	0.37[a]	-
trans-Cypermethrin	-	0.57[b,c]	-	0.39[b,c]	-
cis-Cypermethrin	0.32 [b,f]	-	-	0.16 [b,f]	-
trans-Cyfluthrin	-	0.35[d]	-	-	0.23[d]
cis-Cyfluthrin	0.27[d]	-	-	-	0.10[d]
Deltamethrin	-	-	0.73[e]	0.15[e]	-
Urinary excretion rates (K_{uri_X}, h^{-1})	0.107	0.107	0.192	0.093	0.137

904 [a]Ratelle et al. (2015b); [b]Ratelle et al. (2015a); [c]Woollen et al. (1992); [d]Leng et al. (1997b); [e]Sams and

905 Jones (2012)

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907 **Table 4. *In vivo* studies with different exposures to pyrethroids conducted in humans.**

Study	Molecule	Route	Dose	Data collected
(Gotoh et al., 1998)	Permethrin	Oral	630 mg (poisoning)	Permethrin in blood
(Ratelle et al., 2015b)	Permethrin	Oral	0.1 mg/kg	*cis*- and *trans*-DCCA, and 3-PBA in urine
(Woollen et al., 1992)	Cypermethrin	Oral	3.3 mg	*cis*- and *trans*-DCCA, and 3-PBA in urine
(Ratelle et al., 2015a)	Cypermethrin	Oral	0.1 mg/kg	*cis*- and *trans*-DCCA, and 3-PBA in urine
(Leng et al., 1997a)	Cyfluthrin	Oral	0.03 mg/kg	*cis*- and *trans*-DCCA, and F-PBA in urine
(Sams and Jones, 2012)	Deltamethrin	Oral	0.01 mg/kg	DBCA and 3-PBA in urine
(Tomalik-Scharte et al., 2005)	Permethrin	Dermal	3 g for 12 hours	Total DCCA in urine
(Woollen et al., 1992)	Cypermethrin	Dermal	31 mg for 8 hours	*cis*- and *trans*-DCCA, and 3-PBA in urine
(Leng et al., 1997b)	Cyfluthrin	Inhalation	160 µg/m^3 for 30 min	*cis*- and *trans*-DCCA, and F-PBA in urine

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910 **Table 5. Toxicokinetic parameters calculated from the *in vivo* studies with different exposures to pyrethroids conducted**
911 **in humans. The predictions correspond to the mean and the 95% interval of confidence of 10,000 Monte Carlo**
912 **simulations.**

	Maximum peak concentration or excretion rate		Time-to-peak levels (h)		Elimination half-life (h)	
	Obs	Predictions	Obs	Predictions	Obs	Predictions
Permethrin oral						
Study by Gotoh et al. (1998) – blood concentration (mg/L)						
cis-PM	0.61	0.64 [0.21 – 1.64]	3	2.3 [1.7 – 3.5]	5.0	3.4 [2.1 – 6.5]
trans-PM	0.25	0.32 [0.10 – 0.86]	3	1.7 [1.2 – 2.5]	3.7	4.6 [2.6 – 9.2]
Study by Ratelle et al. (2015b) – urinary concentration (mg/L)						
cis-DCCA	0.61	0.29 [0.09 – 0.57]	[3 – 6]	7.3 [5.2 – 11.0]	6.2	7.8 [4.4 – 16.2]
trans-DCCA	1.22	0.66 [0.21 – 1.26]	[3 – 6]	8.1 [5.7 – 13.0]	6.5	8.0 [4.7 - 16.3]
3-PBA	2.20	1.04 [0.44 – 1.75]	[9 – 12]	8.1 [6.0 – 12.0]	6.7	8.9 [5.1 - 18.3]
Permethrin dermal						
Study by Tomalik-Scharte et al. (2005) – excretion rate (mg/h)						
Total DCCA	0.14	0.20 [0.06 – 0.41]	[18 – 24]	24.0 [19.5 – 32.0]	22.4	19.8 [11.1 - 40.9]
Cypermethrin oral						
Study by Woollen et al. (1992) – excretion rate (µg/h)						
cis-DCCA	9.8	9.3 [2.6 – 18.8]	[0 – 4]	6.7 [4.8 – 10.2]	7.6	8.4 [4.6 – 17.6]
trans-DCCA	16.2	14.7 [4.7 – 28.0]	[0 – 4]	8.1 [5.7 – 12.8]	8.8	10.3 [5.7 – 18.4]
3-PBA	9.3	13.9 [5.6 – 24.5]	[4 – 8]	7.8 [5.8 – 11.3]	9.4	11.1 [6.2 – 20.9]
Study by Ratelle et al. (2015a) – urinary concentration (mg/L)						
cis-DCCA	0.43	0.25 [0.07 – 0.51]	[3 – 6]	6.7 [4.8 – 10.0]	6.7	7.6 [4.1 – 16.4]
trans-DCCA	0.89	0.60 [0.19 – 1.13]	[3 – 6]	8.1 [5.7 – 13.0]	6.4	8.0 [4.6 – 16.5]
3-PBA	0.71	0.50 [0.20 – 0.91]	[12 – 24]	7.9 [5.8 – 12.0]	6.9	8.9 [5.1 – 18.1]
Cypermethrin dermal						
Study by Woollen et al. (1992) – excretion rate (µg/h)						
cis-DCCA	0.60	0.23 [0.05 – 0.50]	[24 – 36]	20.0 [15.2 – 30.0]	13.9	17.5 [10.0 – 37.2]
trans-DCCA	0.63	0.32 [0.08 – 0.68]	[12 – 24]	20.0 [15.3 – 30.0]	15.4	17.6 [10.0 – 37.2]
3-PBA	3.84	0.33 [0.10 – 0.68]	[12 – 24]	21.0 [15.8 – 31.0]	7.9	18.1 [10.3 – 37.6]
Cyfluthrin inhalation						
Study by Leng et al. (1997b) – excretion rate (µg/h)						
cis-DCCA	0.25	0.16 [0.05 - 0.31]	[0 – 1]	1.1 [0.7 - 2.0]	6.9	7.5 [4.1 – 15.8]
trans-DCCA	0.38	0.29 [0.08 - 0.55]	[0 – 1]	1.1 [0.7 - 2.0]	6.2	7.5 [4.2 – 15.6]
F-PBA	0.35	0.34 [0.12 - 0.61]	[1 – 2]	0.9 [0.5 - 1.7]	5.3	5.9 [3.3 – 12.1]
Cyfluthrin oral						
Study by Leng et al. (1997a) – excretion rate (µg/h)						
cis-DCCA	3.3	5.0 [1.5 – 10.0]	[0 – 12]	6.6 [4.8 – 10.0]	6.7	8.3 [4.6 – 17.1]
trans-DCCA	11.4	7.9 [2.2 – 15.4]	[0 – 12]	8.0 [5.5 – 12.8]	6.6	10.3 [5.7 – 18.1]
F-PBA	21.1	8.9 [3.5 – 15.9]	[0 – 12]	6.7 [5.0 – 9.8]	6.2	8.2 [4.6 – 16.5]
Deltamethrin oral (µg/L)						
Study by (Sams and Jones (2012)) – urinary concentration (mg/L)						
DBCA	0.59	0.28 [0.09 - 0.51]	[0 – 2]	4.5 [3.2 – 6.8]	4.0	4.7 [2.6 – 9.8]
3-PBA	0.06	0.04 [0.01 - 0.07]	[2 – 4]	5.7 [4.0 – 9.0]	8.3	8.9 [5.0 – 18.2]

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916 **Table 6. Calculated exposures of the French adult population to pyrethroids by the three routes of exposure, *i.e.* ingestion, inhalation and dermal contact. The results are represented by the average value ± SD.**

Compound	Oral (µg/kg/d)	Inhalation (µg/m³)	Dermal (µg/d)
Permethrin			
total	0.020 ± 0.100	$7.3 \times 10^{-5} \pm 3.7 \times 10^{-4}$	0.486 ± 2.430
cis isomer	0.008 ± 0.040	$2.9 \times 10^{-5} \pm 1.5 \times 10^{-4}$	0.194 ± 0.970
trans isomer	0.012 ± 0.060	$4.4 \times 10^{-5} \pm 2.2 \times 10^{-4}$	0.292 ± 1.460
Cypermethrin			
total	0.015 ± 0.075	$1.9 \times 10^{-5} \pm 9.5 \times 10^{-5}$	0.093 ± 0.465
cis isomer	0.006 ± 0.030	$0.8 \times 10^{-5} \pm 4.0 \times 10^{-5}$	0.039 ± 0.195
trans isomer	0.009 ± 0.045	$1.1 \times 10^{-5} \pm 5.5 \times 10^{-5}$	0.054 ± 0.270
Cyfluthrin			
total	0.012 ± 0.060	$6.0 \times 10^{-6} \pm 3.0 \times 10^{-5}$	0.029 ± 0.145
cis isomer	0.005 ± 0.025	$2.0 \times 10^{-6} \pm 1.0 \times 10^{-5}$	0.012 ± 0.060
trans isomer	0.007 ± 0.035	$4.0 \times 10^{-6} \pm 2.0 \times 10^{-5}$	0.017 ± 0.085
Deltamethrin	0.015 ± 0.075	$7.0 \times 10^{-6} \pm 3.5 \times 10^{-5}$	0.029 ± 0.145

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