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1 **Estimating human exposure to pyrethroids' mixtures from biomonitoring**
2 **data using physiologically based pharmacokinetic modeling**

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17 **Highlights**

- 18 • We aimed at estimating the exposure of a French cohort to pyrethroids' mixtures
- 19 • Cumulative exposures were estimated from the urinary metabolites' concentrations
- 20 • The permethrin-cypermethrin pair, cyfluthrin and deltamethrin contributed equally
- 21 • Variability in metabolism enabled to explain some of the variations in the data
- 22 • No risks for three neurotoxicity endpoints were associated with the mixtures

23

24

25 **Abstract**

26 Human biomonitoring data provide evidence to exposure of environmental chemicals. Physiologically
27 based pharmacokinetic (PBPK) modelling together with an adequate exposure scenario allows to
28 transpose measured concentrations of chemicals or their metabolites into exposure levels, as daily
29 intakes. In France, high levels of urinary pyrethroids metabolites have been measured in populations.
30 Our work aims at estimating the exposure of the French ENNS cohort to mixtures of four pyrethroids
31 (deltamethrin, permethrin, cypermethrin, and cyfluthrin) from the urinary concentrations of five
32 pyrethroids' metabolites commonly measured in biomonitoring studies. We developed a modelling
33 approach based on a global toxicokinetic model that accounts for the cumulative exposure to
34 pyrethroids as some of the metabolites can be shared by several parent compounds and for human
35 inter-individual variability in metabolism. The median of the individual daily intakes was estimated to
36 8.1 ng/kg bw/day for permethrin, 17.7 ng/kg bw/day for cypermethrin, 20.4 ng/kg bw/day for
37 cyfluthrin and 34.3 ng/kg bw/day for deltamethrin leading to similar weights for the pair permethrin
38 and cypermethrin (36%), cyfluthrin (31%) and deltamethrin (33%) to the cumulative exposure.
39 Accounting for human variability enabled to explain some of the variations in the metabolites' levels
40 within the cohort. The cumulative exposure was then weighted by their toxicities towards three
41 neurotoxic effects to calculate margins of exposure (MOE). Low MOE values were always associated
42 with high measured concentrations of metabolites in urine and the lowest MOEs were observed for the
43 autonomic division. No risks associated with reconstructed mixtures of pyrethroids were expected for
44 the ENNS cohort. Our approach is an asset to analyse the biomarkers of exposure to pyrethroids
45 simultaneously and could be easily adapted to any local or national specificities in pyrethroids'
46 exposure or populations.

47

48 **Keywords:** PBPK model, pyrethroids, reverse dosimetry, cumulative risk, mixtures.

49

50 **1 Introduction**

51 Human biomonitoring (HBM) data reflect the body burden of chemicals or a biological effect resulting
52 from exposures via different sources and routes (CDC, 2009). HBM data can then refer to the
53 measurement of chemicals or their metabolites in human tissues or biofluids, such as blood or urine.
54 Their interpretation in a population health risk context is now facilitated by the availability of
55 biomonitoring screening values, such as biomonitoring equivalents (BEs) or HBM values, to which
56 the HBM data can be directly compared (Hays and Aylward, 2009; Angerer *et al.*, 2011). These BEs
57 or HBM values are defined as the concentration of a chemical (a parent compound or metabolite) in a
58 biological medium (blood, urine, human milk, etc.) consistent with existing exposure guidance values
59 such as reference doses or concentrations, or acceptable or tolerable daily intakes (Hays *et al.*, 2007).

60 Over the last two decades, numerous HBM studies have shown the wide exposure of the general
61 population to pyrethroids (Barr *et al.*, 2010; Babina *et al.*, 2012; Roca *et al.*, 2014). Pyrethroids share
62 the same insecticidal mode of action (*i.e.*, disrupting neuronal function by binding to voltage-gated
63 sodium channels), and are usually divided into two types (type I and type II) depending on their
64 chemical structure (absence or presence of an α -cyano group) and the undesirable symptoms they
65 cause. Type I pyrethroids have been reported to cause the tremor type syndrome (T), aggressive
66 behavior, hypersensitivity and ataxia, and type II pyrethroids salivation, the choreoathetosis-salivation
67 syndrome (CS) and motor dysfunction in mammals (Ray and Forshaw, 2000; Soderlund, 2012;
68 Chrustek *et al.*, 2018). Other effects, like oxidative stress or effects on male fertility and prenatal
69 development, have also been reported (Saillenfait *et al.*, 2015; Wang *et al.*, 2016; Lu *et al.*, 2019). In
70 HBM studies, exposure to pyrethroids is usually monitored *via* five metabolites excreted in urine: 3-
71 phenoxybenzoic acid (3-PBA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-
72 carboxylic acid (*cis*-DCCA and *trans*-DCCA), 4-fluoro-3-phenoxybenzoic acid (F-PBA), 3- (2,2-
73 dibromovinyl) -2,2- dimethyl cyclopropane carboxylic acid (DBCA). DBCA is a metabolite specific to
74 deltamethrin, and F-PBA to cyfluthrin but can be formed from the two isomers (Figure 1). *Cis*- and
75 *trans*-DCCA can be formed respectively from the *cis* and *trans* isomers of permethrin, cypermethrin,
76 and cyfluthrin. The metabolite 3-PBA is common to numerous pyrethroids, including permethrin,

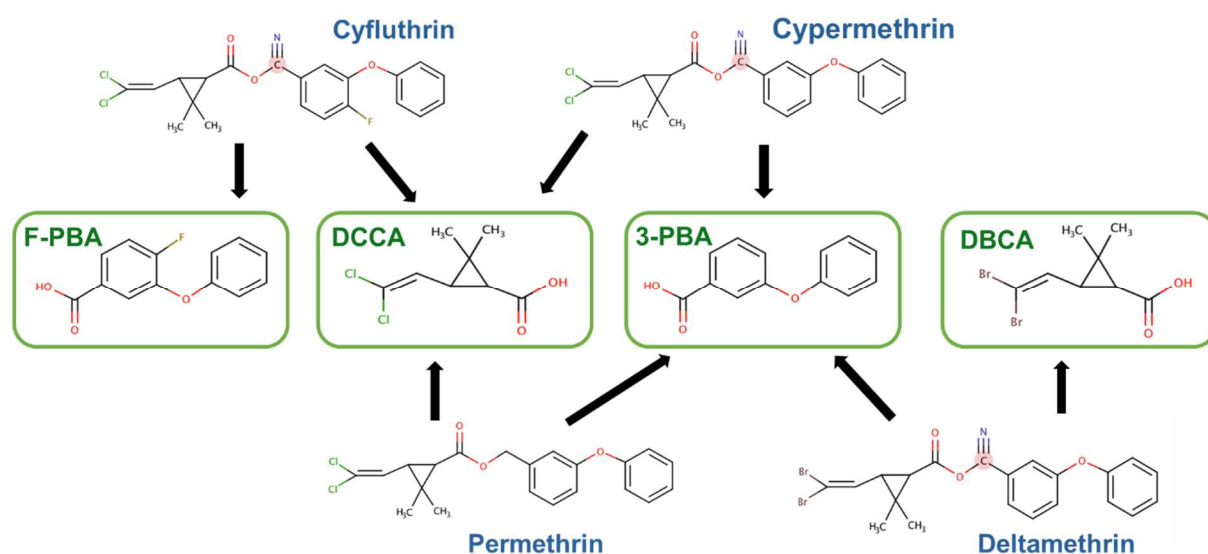
77 cypermethrin and deltamethrin. BEs were derived for three of five metabolites: F-PBA specific to
78 cyfluthrin (Hays *et al.*, 2009), DBCA specific to deltamethrin (Aylward *et al.*, 2011), and 3-PBA
79 shared by to numerous pyrethroids (Aylward *et al.*, 2018). The derivation of a BE for a metabolite
80 shared by several compounds is not straightforward, especially if the toxicity differs between the
81 parent compounds. Recently, Aylward *et al.* (2018) developed an original approach in two tiers for the
82 common metabolite 3-PBA: the first tier is based on the steady-state urinary concentrations of 3-PBA
83 associated with an exposure guidance value (Reference dose or Acceptable Daily Intake) for each of
84 the nine pyrethroids included in their study and assumes that all of the measured 3-PBA arises from
85 the compound with the lowest BE value (*i.e.*, deltamethrin and cyhalothrin). The second tier is based
86 on weighting by relative exposure estimates for the different pyrethroid compounds. The first-tier
87 value can be used as a conservative screening value, and the authors recommend applying the second
88 tier when the HBM data exceed the tier 1 value.

89 In France, the most up-to-date biomonitoring study for the general French population (National Health
90 Nutrition Study (ENNS) in 2006-2007) showed high levels of pyrethroids compared to other European
91 or American populations (Fréry *et al.*, 2011). Recently, the ELFE cohort showed the same trends in
92 pregnant women (Dereumeaux *et al.*, 2018). Regarding the ENNS cohort, the measured urinary
93 concentrations of F-PBA and DBCA are well below the existing BEs but about 15% of the participants
94 have their 3-PBA urinary concentrations exceeding the tier 1 value (1.7 µg/L) proposed by Aylward *et*
95 *al.* (2018). The second tier of their approach is not directly applicable to the French population as it
96 involves exposure estimates of the pyrethroids for the American population that are not relevant for
97 the French population, as the uses of pyrethroids and the national regulations differ. An alternative
98 approach to the use of BE values would be to estimate the exposure, in the form of a daily intake for
99 example, from HBM data and to compare the estimated exposures to guidance values (Clewell *et al.*,
100 2008; Zeman *et al.*, 2013; Sarigiannis *et al.*, 2019b). In that context, physiologically based
101 pharmacokinetic (PBPK) models can be used to link an external exposure to a chemical to internal
102 dosimetry by accounting for its absorption, distribution, metabolism and excretion, and for the
103 anatomy and physiology of the individual (Bois and Brochot, 2016; Sarigiannis *et al.*, 2019a).

104 Recently, we developed a global toxicokinetic model that links the urinary concentrations of the five
105 metabolites measured in HBM studies to the external exposure of the four pyrethroids, i.e.
106 deltamethrin, permethrin, cypermethrin, and cyfluthrin (Figure 1). Our model includes physiologically
107 based pharmacokinetic models (PBPK) for the parent compounds (or their isomers *cis* and *trans*) and
108 one-compartment models for the metabolites (Quindroit *et al.*, 2019). The development of the models
109 was based on experimental toxicokinetic studies in mammals (rats and humans) that identified
110 common features: significant and rapid oral absorption, rapid distribution in the tissues and organs,
111 accumulation in fat, and high hepatic metabolism with short half-lives in blood (e.g., Anadon *et al.*,
112 1991; Anadon *et al.*, 1996; Leng *et al.*, 1997a; Godin *et al.*, 2010; Tornero-Velez *et al.*, 2012; Ratelle
113 *et al.*, 2015a; Ratelle *et al.*, 2015b; Willemin *et al.*, 2016). The global model aims at aggregating the
114 exposures via different sources and routes, and at calculating the cumulative amount of the five
115 metabolites from the four parent compounds.

116 In this work, we estimated the exposure to deltamethrin, permethrin, cypermethrin, and cyfluthrin of
117 the French ENNS cohort from the HBM data with our global model for pyrethroids. Given the
118 estimated exposures, we assessed the risk related to three neurotoxic effects of the reconstructed
119 pyrethroids' mixtures using the methodology on cumulative assessment groups of pesticides by EFSA
120 (2019a).

121



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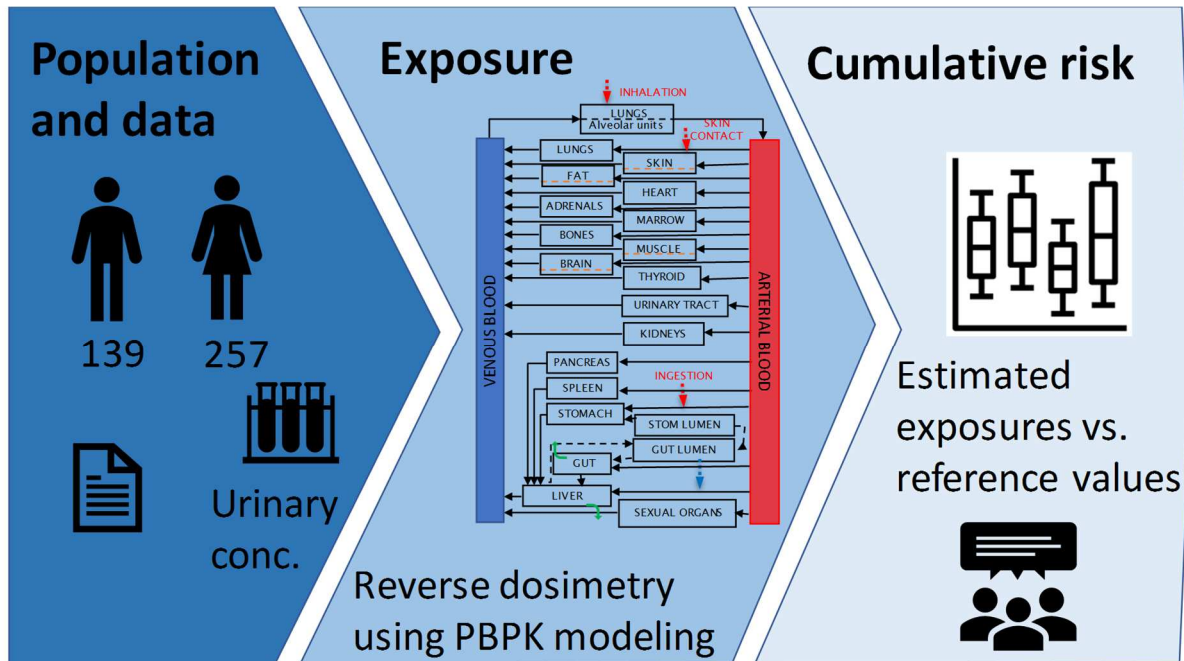
123 **Figure 1: Pyrethroids and their urinary metabolites measured in biomonitoring studies, adapted**
 124 **from Ueyama et al. (2010) and Starr et al. (2008)**

125

126 **2 Materials and Methods**

127 The proposed approach is based on the population and the HBM data of the ENNS cohort, the
 128 assessment of the pyrethroids' exposures using reverse dosimetry with PBPK modelling, and the
 129 assessment of the cumulative risk from the reconstructed exposures (Figure 2).

130



131

132 **Figure 2: Workflow of our approach to estimate the exposure and the cumulative risk to**
 133 **pyrethroids for the ENNS cohort.**

134 **2.1 Study population**

135 The Nutrition & Health Survey (ENNS study) was carried out by the French Institute for Public Health
 136 Surveillance (InVS) in 2006-2007 (Fréry et al., 2011). The ENNS study was launched in the
 137 framework of the National Program on Nutrition & Health implemented in France in 2001. One of the
 138 aims was to describe the exposure of the French population to few heavy metals and pesticides and to
 139 identify their determinants of exposure. A total of 396 adults (257 women and 139 men) aged 18 to 74
 140 years were enrolled in the pyrethroids' study. Individual characteristics, as age, bodyweight, and food

141 questionnaires (with the consumption of several food groups like fruits, vegetables, cereals, meat and
142 fish), were collected for each study participant. First morning voids were collected to measure the
143 concentrations of the pyrethroids' metabolites.

144 **2.2 Chemicals and biomonitoring data**

145 The four pyrethroids considered in this study were: cyfluthrin (type II), cypermethrin (type II),
146 deltamethrin (type II), and permethrin (type I). The urinary concentrations of five pyrethroids'
147 metabolites were measured in the first morning voids of the 396 participants: *cis*- and *trans*-DCCA
148 ($C_8H_{10}Cl_2O_2$, molecular weight = 209.07 g/mol, CAS numbers: 55701-05-8 and 55701-03-6), DBCA
149 ($C_8H_{10}Br_2O_2$, molecular weight = 297.97 g/mol, CAS number: 63597-73-9), 3-PBA ($C_{13}H_{10}O_3$,
150 molecular weight = 214.22 g/mol, CAS number: 3739-38-6), and F-PBA ($C_{13}H_9FO_3$, molecular weight
151 = 232.21 g/mol, CAS number: 77279-89-1). The urinary concentrations of the pyrethroids'
152 metabolites were measured by high performance liquid chromatography with the limit of
153 quantification (LOQ) set to 0.1 $\mu\text{g/L}$ (Fréry et al., 2011). Table 1 presents the validation criteria of the
154 analytical method used by Fréry *et al.* (2011). The method for measuring pyrethroids' metabolites in
155 the urine was considered reliable regarding the three criteria reported: the precision in the series was
156 about 3%, the inter-day precision ranged between 6% and 9%, and the recovery lied between 78% to
157 103%. Four women were excluded of the dose reconstruction analysis as their metabolites'
158 measurements were all below the LOQ. A summary of the HBM data is presented in Table 2.

159 The relationships between the measurements of the five urinary metabolites' concentrations were
160 investigated using several statistical tests to identify internal exposure patterns. First, we tested if the
161 non-quantification of a certain metabolite could impact the quantification of the others. Then we tested
162 the relationships between the quantified samples. Post hoc tests were conducted (Student's t-test or
163 Tukey's range test) using R CRAN (<https://cran.r-project.org/>).

164

165 **Table 1: Validation criteria of the analytical method used for measuring the pyrethroids'**
 166 **metabolites in the urine of the ENNS participants as reported by Fréry et al. (2011).**

Compound	LOD (µg/L)	LOQ (µg/L)	Precision in the series (CV %)	Inter-day precision (CV %)	Recovery (%)
cis-DCCA	0.03	0.1	1.7	7.8 (0.9)	88
trans-DCCA	0.03	0.1	2.8	8.8 (0.9)	78
DBCA	0.03	0.1	2.6	7.0 (1.0)	82
F-PBA	0.03	0.1	1.7	6.3 (1.0)	103
3-PBA	0.03	0.1	1.9	6.3 (1.8)	93

167
168

169 **Table 2: Summary of individual characteristics and the urinary concentrations of the**
 170 **pyrethroids' metabolites (µg/L) for the ENNS participants (n total = 396, n women = 257, n men**
 171 **= 139). The geometric mean, the standard deviation and the 95% interval are reported.**

	Men	Women
Age (y)	45.2±14.5 [20.0; 72.6]	44.9 ± 13.3 [24.0; 72.0]
Bodyweight (kg)	76.9±12.7 [57.0; 103.4]	63.9 ± 15.0 [44.1; 104.4]
Urinary concentrations* (µg/L)		
<i>cis</i> -DCCA	0.13±0.53 [<LOQ; 1.43]	0.14±1.48 [<LOQ; 3.32]
<i>trans</i> -DCCA	0.33±1.49 [<LOQ; 3.88]	0.33±4.56 [<LOQ; 7.28]
DBCA	0.38±2.36 [<LOQ; 4.36]	0.29±1.94 [<LOQ; 3.73]
F-PBA	<LOQ [<LOQ; 1.29]	<LOQ [<LOQ; 1.74]
3-PBA	0.67±1.29 [0.15; 4.62]	0.65±4.23 [0.11; 7.52]
Quantification of metabolites		
All metabolites quantified	20 (15%)	43 (17%)
4 metabolites quantified	67 (48%)	105(41%)
3 metabolites quantified	35 (25%)	61 (24%)
2 metabolites quantified	13 (9%)	35 (14%)
1 metabolite quantified	4 (3%)	9 (3%)
No metabolites quantified	0 (0%)	4 (1%)
Frequency of quantification (%)		
<i>cis</i> -DCCA	55	57
<i>trans</i> -DCCA	88	85
DBCA	89	80
F-PBA	31	29
3-PBA	99	98

172 *here, the non-quantified samples were set to the LOQ/2 (i.e., 0.05 µg/L)

173

174 2.3 Assessment of the exposures to pyrethroids

175 2.3.1 PBPK model for pyrethroids

176 To simulate the toxicokinetic of the pyrethroids in humans, we used our global toxicokinetic model
 177 that links the external exposure to four pyrethroids and their isomers to the urinary concentrations of
 178 their metabolites (*cis*- and *trans*-DCCA, 3-PBA, F-PBA and DBCA) (Quindroit *et al.*, 2019). This

179 global model is the combination of seven PBPK models for the parent compounds (*cis* and *trans*
180 isomers of permethrin, cypermethrin, cyfluthrin, plus deltamethrin) and five one-compartment models
181 for the metabolites. The *cis* and *trans* isomers were treated separately as their toxicokinetics are known
182 to be different in mammals (e.g., Tornero-Velez *et al.*, 2012; Willemin *et al.*, 2016; Pang *et al.*, 2020),
183 Because 3-PBA can be produced by other pyrethroids than the four of interest in this study, an
184 additional intake was defined in the central compartment of the 3-PBA model. The global model was
185 parameterized with animal and human *in vitro* and *in vivo* data, as well as *in silico* predictions (e.g.,
186 for the partitioning into the tissues). Except for physiological parameters, the global model was similar
187 between men and women. The bodyweight and the age of each participant were used to individualize
188 the PBPK models.

189 2.3.2 *Exposure scenario*

190 Diet was the only exposure source considered as several studies in France, but also in other countries,
191 showed that the oral route via food is the main source of exposure to pyrethroids (Schettgen *et al.*,
192 2002; Riederer *et al.*, 2008; Darney *et al.*, 2018; Vanacker *et al.*, 2020).

193 Because cypermethrin and permethrin share the same metabolites among the measured ones (*cis* or
194 *trans*-DCCA and 3-PBA), their respective exposure cannot be determined solely from the urinary
195 metabolites' concentrations. We then used a ratio to apportion the dietary exposures between the
196 permethrin's intake and the cypermethrin's one. First, the diet permethrin and cypermethrin intakes
197 were calculated for each ENNS participant based on his/her food questionnaire, by multiplying the
198 food consumption by the concentrations of the two compounds measured in French food. These
199 concentrations came from average food residue data measured in contamination control and food
200 monitoring surveys carried out in France between 2007 and 2013. Only samples above the limit of
201 quantification were used to compute the intakes. For each participant, we then obtained an individual
202 ratio of his/her computed dietary intakes (PM/CYP ratio).

203 To reconstruct the pyrethroids' exposures from the HBM data, we considered a continuous exposure
204 *via* oral intake for each parent compound in order to reach a steady-state. The daily intakes for
205 cypermethrin, cyfluthrin and deltamethrin were estimated from the HBM data, and the daily intake for

206 permethrin was computed from the cypermethrin intake and the PM/CYP ratio. As no information was
207 available on isomeric ratios of pyrethroids, the ratios usually observed in commercial formulations
208 were used to compute the isomers' intakes, *i.e.* 40:60 *cis:trans* for permethrin and cyfluthrin, and
209 42:58 for cypermethrin.

210 2.3.3 Bayesian analysis

211 A Bayesian approach was applied to estimate the intakes of the pyrethroids from the individual urinary
212 concentrations of five pyrethroids' metabolites of the ENNS cohort. Reverse dosimetry simulations
213 were performed to scale the diet intakes of the parent compounds to the measured urinary
214 concentrations of metabolites. The reverse dosimetry approach was based on the Bayes' formula:

$$215 \quad P(DI|U) = \frac{P(U|DI)}{\sum[P(U|DI_i)]}$$

216 where DI is the diet intake of the parent compound, U the measured urinary concentrations, $P(DI|U)$
217 the probability of a particular daily intake given the measured urinary concentrations, $P(U|DI)$ the
218 probability of the urinary concentration predicted by the PBPK model at a given intake.

219 For each ENNS participant, a non-informative (uniform) *prior* distribution was assigned to the
220 deltamethrin, cypermethrin, and cyfluthrin intakes and to the additional 3-PBA intake. Some
221 toxicokinetic parameters varied among the ENNS participants to allow inter-individual variability and
222 to estimate the impact of that variability on the intakes' estimates. We selected these parameters using
223 the results of a global sensitivity analysis that was performed on the global model and that showed that
224 the most influent parameter for each urinary metabolite concentration was the fraction of parent
225 compound that is transformed into that metabolite (Quindroit *et al.*, 2019). Truncated normal prior
226 distributions were assigned to these fractions of metabolites with a coefficient of variation of 30%.
227 The lower bound was set to 0.01% of the mean value of the parameter and the upper bound was
228 truncated at 1 (Table 3).

229 An error model was defined for the urinary concentrations of metabolites, that were assumed to follow
230 a lognormal distribution with 15% of error. To account for the concentrations below the LOQ, a

231 statistical model was defined. The likelihood of the samples below the LOQ was computed as the
 232 cumulative normal density function (from $-\infty$ to the LOQ, *i.e.* 0.1). For each unquantified sample, this
 233 model will assign a concentration below the LOQ consistent with the other metabolite concentrations
 234 of the ENNS participant.

235 Markov Chain Monte Carlo simulations were used to estimate the posterior probability distributions
 236 using MCSim (Bois and Maszle, 1997). Three independent Markov Chains of 14,000 iterations were
 237 run and one in two of the last 4,000 iterations were recorded. The convergence of the chains was
 238 checked with the \hat{R} criterion (Gelman *et al.*, 1995).

239

240 **Table 3: Prior distributions of the fraction of metabolites formed by a parent compound for the**
 241 **Bayesian analysis. The mean, standard deviation and truncation bounds are reported.**

Fraction of metabolites	Distributions	Truncation
<i>trans</i> -Permethrin		
<i>trans</i> -DCCA	Normal(0.61,0.18)	[0.12-1.00]
3-PBA	Normal(0.85,0.26)	[0.17-1.00]
<i>cis</i> -Permethrin		
<i>cis</i> -DCCA	Normal(0.37,0.11)	[0.07-1.00]
3-PBA	Normal(0.37,0.11)	[0.07-1.00]
<i>trans</i> -cypermethrin		
<i>trans</i> -DCCA	Normal(0.57,0.17)	[0.11-1.00]
3-PBA	Normal(0.39,0.12)	[0.08-1.00]
<i>cis</i> -Cypermethrin		
<i>cis</i> -DCCA	Normal(0.32,0.10)	[0.06-1.00]
3-PBA	Normal(0.16,0.05)	[0.03-1.00]
<i>trans</i> -Cyfluthrin		
<i>trans</i> -DCCA	Normal(0.35,0.11)	[0.07-1.00]
F-PBA	Normal(0.23,0.07)	[0.05-1.00]
<i>cis</i> -Cyfluthrin		
<i>cis</i> -DCCA	Normal(0.27,0.08)	[0.05-1.00]
F-PBA	Normal(0.10,0.03)	[0.02-1.00]
Deltamethrin		
DBCA	Normal(0.73,0.22)	[0.15-1.00]
3-PBA	Normal(0.15,0.05)	[0.03-1.00]

242

243 2.4 Cumulative risk assessment

244 To compute the cumulative risk assessment of the mixture of the four pyrethroids of interest, we
245 employed the method proposed by Vanacker *et al.* (2020) based on the EFSA publication on
246 cumulative assessment groups (CAGs) of pesticides for their effects on the nervous system (EFSA,
247 2019a). This methodology assumes that all compounds included in a CAG combine their effects by
248 dose addition, which has been showed for mixtures of type II pyrethroids *in vitro* and in animals
249 (Wolansky *et al.*, 2009; Cao *et al.*, 2011; Romero *et al.*, 2015). Cyfluthrin, cypermethrin, deltamethrin
250 and permethrin are all included in the three CAGs for functional alterations of the motor division, the
251 sensory division and the autonomic division in chronic exposure/risk assessments. Table 4 summarizes
252 the no observed adverse effect levels (NOAEL) and the toxicological endpoints (indicators of specific
253 effect) retained by EFSA (2019a) for the four parent compounds. Under the additivity hypothesis, the
254 cumulative exposure to the four pyrethroids was calculated using the relative potency factor (RPF)
255 approach (U.S. EPA, 2011; EFSA, 2019b). Regarding a common toxicity effect, the RPF of each
256 substance in a CAG was calculated by dividing the toxicity reference point of the substance (NOAEL)
257 by those of the index compound (here, deltamethrin). For each of the three neurotoxic effects, the
258 cumulative exposure (E_{cumul}) was calculated as the sum of the estimated daily intakes (DI) for each
259 pyrethroid (x) multiplied by its RPF (Table 4):

$$260 \quad E_{cumul} = \sum_x (DI_x \times RPF_x)$$

261 and the risk was estimated by calculating the margin of exposure (MOE) by dividing the NOAEL of
262 deltamethrin (Table 4) by the cumulative exposure to the four pyrethroids:

$$263 \quad MOE = NOAEL_{DLT} / E_{cumul}$$

264 Considering a default uncertainty factor of 100 (multiplying 10 to account for interspecies differences
265 by 10 for intraspecies variability), if the MOE is lower than 100, the risk related to the specific
266 neurotoxic effect resulting from the exposure to the mixture of the four pyrethroids cannot be
267 excluded.

268 To assess the cumulative risk of the French cohort, we first computed the cumulative pyrethroids
 269 exposure weighted by their RPFs for the three neurotoxic endpoints. For each participant, the 6,000
 270 MCMC vectors were used to provide a distribution of these weighted exposures. These individual
 271 distributions were then used to compute a distribution for the three MOEs. For each participant, the 5th
 272 percentile of his/her MOEs' distribution was selected to be compared to the threshold of 100.

273

274 **Table 4: NOAELs and the corresponding toxicological endpoints of the four pyrethroids for the**
 275 **EFSA CAGs on functional alterations of the motor division, the sensory division and the**
 276 **autonomic division to be used in chronic exposure/risk assessments (EFSA, 2019a). Deltamethrin**
 277 **was the reference compound for calculating the relative potency factors (RPF).**

Substances	NOAEL mg/kg bw/d	RPF	Toxicological endpoints
Motor division			
Cyfluthrin	2.4	0.417	Ataxia
Cypermethrin	5	0.200	Ataxia, tremor
Deltamethrin	1	1.000	Ataxia, landing-foot, splay, tremor
Permethrin	40	0.025	Increased motor activity, tremor
Sensory division			
Cyfluthrin	0.3	13.30	- (no chronic neurotoxicity studies available, a pharmacological study was used as surrogate)
Cypermethrin	5	0.80	Hypersensitivity to noise
Deltamethrin	4	1.00	Hypersensitivity to noise
Permethrin	100	0.04	Hypersensitivity
Autonomic division			
Cyfluthrin	0.3	3.333	- (no chronic neurotoxicity studies available, a pharmacological study was used as surrogate)
Cypermethrin	6	0.167	Salivation
Deltamethrin	1	1.000	Mydriasis
Permethrin	100	0.010	Piloerection

278

279

280

281

282 **3 Results**

283 **3.1 Statistical analyses of the biomonitoring data**

284 Shapiro tests showed that the urinary metabolites' concentrations for both men and women were log-
285 normally distributed (p values < 0.001). All the statistical tests were then performed with the log-
286 normal transformed concentrations. An ANOVA showed that there was no significant difference in the
287 distribution of urinary metabolites' concentrations between men and women in the ENNS cohort. As
288 the levels of quantification in the urinary samples varied between the five metabolites (from 29.8% for
289 *cis*-DCCA to 98.5% for 3-PBA), we carried out several statistical tests on the relationships between
290 the metabolites' levels in urine. The independence of the quantification of the metabolites in urine was
291 tested using Pearson's Chi-square tests. Only 3-PBA was not included in the analyses as the number of
292 non-quantified samples was very low among the participants (6 over 396). Our results showed that *cis*-
293 DCCA, *trans*-DCCA and DBCA were most often quantified in the same samples ($p < 0.05$). F-PBA
294 non-quantification was independent of the three other metabolites ($p > 0.05$). ANOVA were then
295 performed to assess the relationships between the metabolites' urinary concentrations by testing if the
296 non-quantification of a metabolite influenced the concentrations of the other metabolites. We observed
297 that non-quantified levels of *cis*-DCCA were associated with low urinary concentrations of *trans*-
298 DCCA ($p < 0.001$), DBCA ($p < 0.001$) and 3-PBA ($p < 0.001$). No impact on F-PBA concentrations
299 was observed. Regarding *trans*-DCCA, the non-quantification was associated with low concentrations
300 of DBCA ($p < 0.02$) and 3-PBA ($p < 0.001$). Similar trends were obtained for non-quantification of
301 DBCA with *trans*-DCCA ($p < 0.01$) and 3-PBA ($p < 0.001$). No associations were identified for F-
302 PBA. The sample sizes of the different groups of non-quantified/quantified metabolites are given in
303 Supplementary Data (Table S1).

304 **3.2 Ratio of exposure between permethrin and cypermethrin**

305 For men, the mean daily intakes estimated using the food questionnaires were 133.9 ng/kg bw/day
306 (CV = 56%) for permethrin and 292.2 ng/kg bw/day (CV = 74%) for cypermethrin. For women, the
307 mean daily intakes were a bit lower: 98.0 ng/kg bw/day (CV = 64%) for permethrin and 223.0 ng/kg
308 bw/day (CV = 64%) for cypermethrin. The individual PM/CYP ratio computed from these exposures

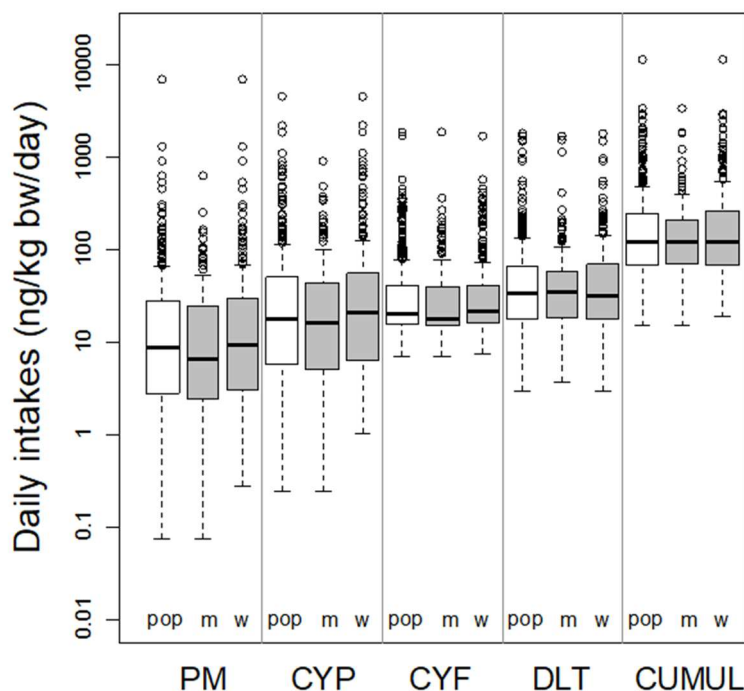
309 resulted in geometric means of 0.49 (SD = 0.31, IC95% [0.12; 1.33]) for men and 0.46 (SD = 0.36,
310 IC95% [0.11; 1.46]) for women. These intakes are in the ranges of other studies for the French
311 population (Darney *et al.*, 2018; Vanacker *et al.*, 2020).

312 **3.3 Estimation of the daily intakes from the urinary concentrations**

313 The global toxicokinetic model was adjusted to the urinary concentrations of the five metabolites to
314 estimate the daily intakes of the seven pyrethroids and isomers for each individual. Four women were
315 not included, as no metabolites were quantified in their urine. Globally, the convergence of the three
316 chains for men and women was reached. First, the model estimates of the urinary concentrations of the
317 five metabolites were checked against the measured concentrations. Several figures show the
318 goodness-of-fit in the Supplementary Data (Figure S1). There was a good agreement between the data
319 and predictions for all metabolites, as all the estimated concentrations lay into the 2-fold error interval.
320 When the median is considered, the estimated DBCA concentrations were very close to the data (less
321 than 3% of deviation), F-PBA concentrations were underpredicted for most of the individuals, and the
322 3-PBA estimates were always higher than the measured concentrations (but no more than a 30%
323 deviation). There were no general trends for *cis*-DCCA and *trans*-DCCA. The model estimates of the
324 concentrations under the LOQ were also checked in details (Figure S2 in Supplementary Data).
325 Precise estimates of the concentrations were obtained for *cis*- and *trans*-DCCA and 3-PBA in men,
326 and for *cis*-DCCA in women. For *trans*-DCCA in women and DBCA and F-PBA in men and women,
327 it was not possible to obtain, for most of the individuals, a precise estimate of the (unquantified)
328 concentration as the 95% confidence interval was estimated between 0 and 0.1 µg/L, that is the LOQ.
329 The uncertainty was high for these metabolites. Nevertheless, for F-PBA, the estimated medians could
330 differ between the individuals (from 0.03 to 0.07 µg/L with an outlier at 4.5 µg/L for a woman). This
331 outlier can be explained by discrepancies in the measured data for that woman: high concentrations of
332 *cis*-DCCA and 3-PBA, and moderate concentrations for *trans*-DCCA. During the calibration process,
333 the optimum was reached by estimating a very high daily intake for cyfluthrin even though the F-PBA
334 concentration was unquantified.

335 The mean daily intakes estimated for the four parent compounds via the exposure reconstruction are
336 provided in Figure 3 for the four parent compounds and in Supplementary Data for the isomers
337 (Figure S3). Regarding the distributions of the mean daily intakes among the whole cohort, the median
338 was estimated to 8.1 ng/kg bw/day for permethrin (IC95% [0.5; 294.9]), 17.7 ng/kg bw/day for
339 cypermethrin (IC95% [1.1; 656.0]), 20.4 ng/kg bw/day for cyfluthrin (IC95% [9.6; 306.7]) and
340 34.3 ng/kg bw/day for deltamethrin (IC95% [4.3; 374.7]). For cypermethrin and permethrin (that was
341 calculated using the cypermethrin's estimate), we can observe that the range of the distributions is
342 wider than for cyfluthrin and deltamethrin with long tails on both sides of the distributions. For these
343 compounds, the maximal daily intake is higher in women than in men (4475.0 vs. 642.5 ng/kg bw/day
344 for permethrin, 4469.3 vs. 917.6 ng/kg bw/day for cypermethrin). The distributions in men and women
345 were compared using Wilcoxon tests because of the non-normality of the distributions of the daily
346 intakes, log transformed or not (checked with Shapiro-Wilk's tests). The Wilcoxon tests showed that
347 the daily intakes were similar between men and women for permethrin (p-value = 0.28), cypermethrin
348 (p-value = 0.14) and deltamethrin (p-value = 0.92), and that they were significantly different for
349 cyfluthrin (p-value = 0.03).

350



352

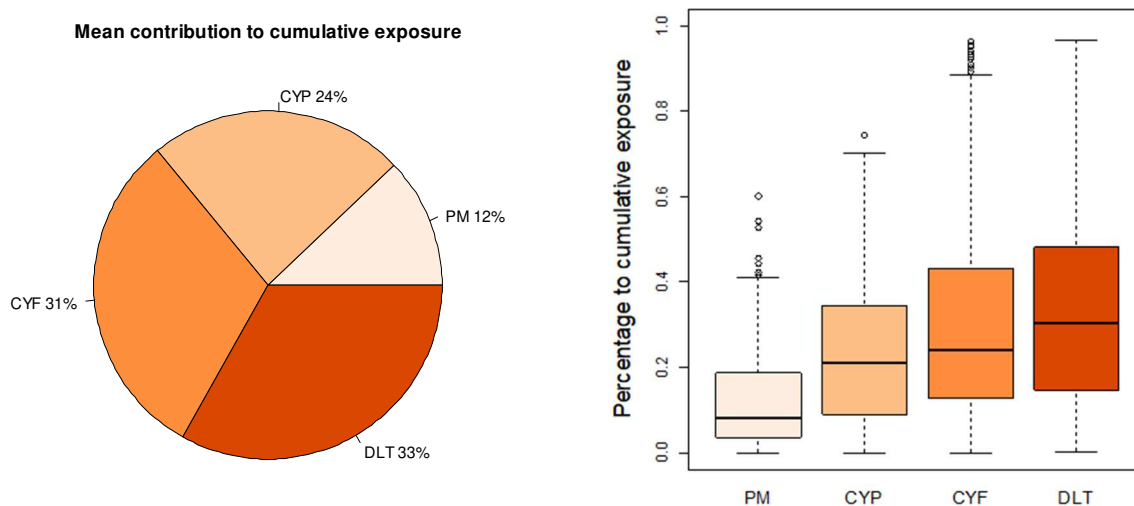
353 **Figure 3: Mean daily intakes for permethrin (PM), cypermethrin (CYP), cyfluthrin (CYF),**
 354 **deltamethrin (DLT) and the cumulative exposure (CUMUL) in the whole population (*pop*), men**
 355 **(*m*) and women (*w*) estimated from the urinary metabolites' concentrations using the global**
 356 **model. The first quartile, the median and the third quartile define the boxplot.**

357

358 The cumulative exposure to pyrethroids was calculated for each individual by summing his/her daily
 359 intakes of permethrin, cypermethrin, cyfluthrin and deltamethrin. The 6,000 MCMC vectors were used
 360 to provide a distribution of intakes for each individual. Figure 3 also presents the distributions of the
 361 individual means for the whole population, as a Wilcoxon test showed no differences between men
 362 and women (mean: p -value = 0.39). The median cumulative exposure of the population was estimated
 363 to 121.6 ng/kg bw/day (IC95 [24.6; 1505.8]). Figure 4 shows the average contribution of the four
 364 pyrethroids to the cumulative daily intake with similar weights for the pair permethrin-cypermethrin
 365 (36%), cyfluthrin (31%) and deltamethrin (33%). However, the contributions can greatly vary within
 366 the population (Figure 4). Indeed, the contribution of cyfluthrin and deltamethrin goes from 0 to 1
 367 meaning that either the individual is not exposed to the compound or that the individual is only

368 exposed to that compound. For permethrin and cypermethrin, the distributions are a bit less wide and
369 varied from 0 to 0.6 (permethrin) or 0.7 (cypermethrin).

370



371 **Figure 4: Mean contribution (left) and distributions of the contributions (right) of each parent**
372 **compound to the pyrethroids' cumulative exposure in the ENNS cohort.**

373

374 Our model also accounts for the 3-PBA production from other pyrethroids than the ones of interest in
375 our study. High inter-individual variability was observed among the cohort, as we found that the
376 proportion (on average) of 3-PBA produced by other sources went from 8% to 96% with a median
377 value at 36%. That proportion was then quite significant for some individuals. On average, our results
378 indicated that permethrin contributed to about 21% of the measured urinary levels of 3-PBA,
379 cypermethrin to 20%, deltamethrin to 18% and other sources to 41% in the ENNS cohort. The
380 correlations of the 3-PBA proportion from other sources with the measured urinary concentrations of
381 the five metabolites were assessed with the Spearman's rank-order correlation. That proportion was not
382 correlated with the urinary concentrations of DBCA and F-PBA, but with the ones of *cis*-DCCA,
383 *trans*-DCCA and 3-PBA. The correlations were low (from -0.13 for 3-PBA to -0.23 for *trans*-DCCA)
384 and negative, *i.e.*, the higher the urinary concentration, the lower the proportion of 3-PBA from other
385 sources.

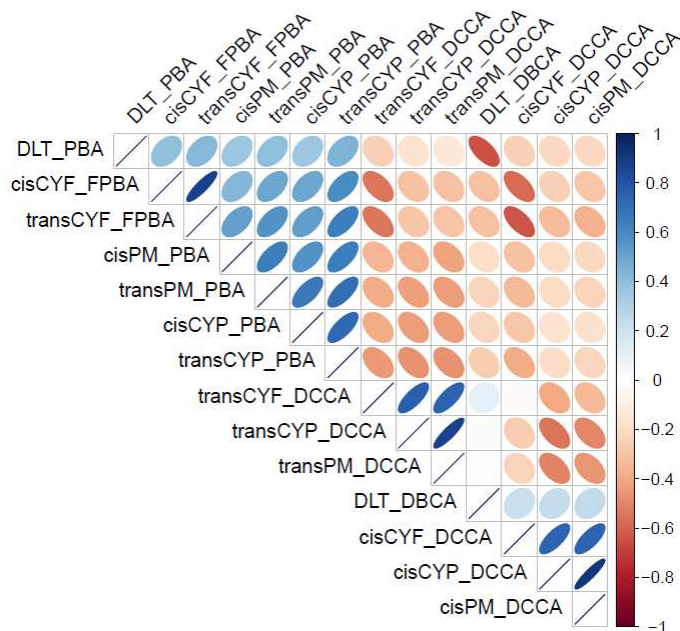
386 3.4 Estimation of the individual parameters

387 Inter-individual variability of the toxicokinetic parameters related to the metabolism was allowed, *i.e.*
388 14 metabolic fractions were estimated for each individual. In most cases, the posterior distributions
389 were similar to the priors (see Figure S4 in Supplementary Data). At the level of the population, this
390 similarity indicates that globally the parameters obtained in laboratory conditions fit quite well with
391 the urinary levels observed in the biomonitoring studies. Deviations from the priors were observed at
392 the population level with posterior distributions lower than prior medians for two parameters: the
393 metabolic fraction of deltamethrin that is transformed into DBCA (prior median: 0.73 and mean of the
394 individual posterior medians: 0.66) and the metabolic fraction of *trans*-permethrin that is transformed
395 to 3-PBA (prior median: 0.85 and mean of the individual posterior medians: 0.72). At the individual
396 level, some posterior distributions deviated significantly from the prior distributions in order to fit the
397 model to the data. We looked at the individuals whose posterior median was not included in the prior
398 interquartile range. About 60% of the population have no posterior distributions outside the prior
399 interquartile range, 20% have one or two posteriors outside the range, 20% have more than two, and
400 2% (4 men and 6 women) have more than four. The metabolic fractions that were the most impacted
401 were: *trans*-CYF into *trans*-DCCA (about 16% of the individuals) and F-PBA (16%), *cis*-CYF into
402 *cis*-DCCA (14%), *trans*-PM into 3-PBA (13%), *cis*-CYP into *cis*-DCCA (13%), and *trans*-CYP into
403 *trans*-DCCA (10%). On the contrary, some metabolic fractions were not affected such as *cis*-PM or
404 *cis*-CYP into 3-PBA, or DLT into 3-PBA and DBCA (even if the posterior medians were lower than
405 the prior one).

406 There are undeniably inter-correlations between the 5 metabolites as they can be formed by the same
407 parent compound. Spearman's rank-order correlation coefficients between the means of the metabolic
408 fraction estimated for each individual were calculated (Figure 5). The fractions producing either F-
409 PBA or 3-PBA were correlated positively such as the ones producing DCCA or DBCA. Conversely,
410 the metabolites produced either by the alcohol (F-PBA and 3-PBA) or acid (DCCA and DBCA) part
411 of the parent compound were correlated negatively. Only few correlations were significant. For
412 instance, the fractions of *trans*- and *cis*-cyfluthrin into F-PBA were highly positively correlated

413 ($\rho=0.88$). Linking by construction the cypermethrin and permethrin intakes (through the PM/CYP
 414 ratios) also affected the correlations of their metabolic fractions into one metabolite, resulting in a
 415 positive correlation between the fractions of cypermethrin into DCCA ($\rho=0.91$ for the *cis* isomer, and
 416 $\rho=0.88$ for the *trans* one) or 3-PBA ($\rho=0.57$ for the *cis* isomer, and $\rho=0.71$ for the *trans* one) with the
 417 corresponding permethrin's ones. The metabolic fractions to form a specific metabolite were generally
 418 correlated ($\rho > 0.5$) with the urinary concentrations of that metabolite. In some cases, a metabolic
 419 fraction of a parent compound was also correlated with the concentration of the second metabolite
 420 formed by the same parent compounds.

421



422

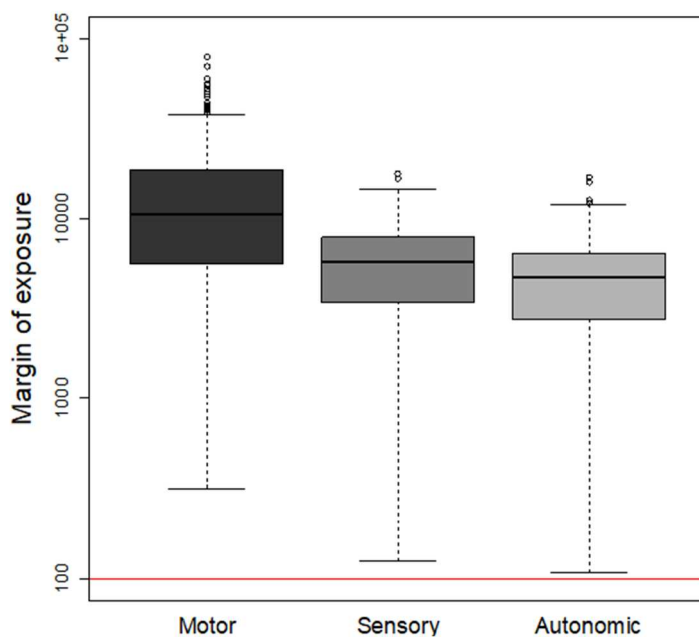
423 **Figure 5: Spearman correlations between the 14 metabolic fractions at the population level. The**
 424 **individual means of the metabolic fraction estimated were used.**

425

426 3.5 Cumulative risk assessment

427 Figure 6 presents the boxplot of the 5th percentile of each individual MOE distribution for the three
 428 neurotoxic effects. Since there were no differences between men and women (checked with Wilcoxon
 429 tests), the whole population was represented on the same graph. All the 5th percentiles of the MOE

430 distributions for all the individuals were above 100 indicating that no risk associated with the
431 cumulative exposure to the four pyrethroids is expected for the ENNS cohort. The median of the
432 population distribution was 10,573 (IC 95% [1,192;44,758]) for motor division, 5,826 (IC 95%
433 [633;12,780]) for sensory division and 4,741 (IC 95% [544;11,107]) for autonomic division. The
434 lowest MOEs were observed for the autonomic division. Eight individuals have a least one of their P5
435 of the three MOEs distributions below 500. These low MOE values were always associated with high
436 measured concentrations of metabolites in urine. We also observed that this is the same person (man)
437 that showed the lowest MOE values for the three toxic effects. This can be explained by his very high
438 levels in urine: the DBCA and 3-PBA concentrations were above the 97.5th percentile of the men
439 distribution, and the F-PBA concentration was the highest one.



440

441 **Figure 6: Distribution of the 5th percentile of the individual distribution of the margin of**
442 **exposure for three neurotoxic effects**

443

444 **4 Discussion**

445 In this work, we developed a modelling approach to interpret the biomarkers of exposure to
446 pyrethroids considering that some of them reflect a cumulative exposure to several parent compounds.
447 Daily intakes to four pyrethroids of the French ENNS cohort were estimated from the urinary
448 concentrations of five metabolites using a global toxicokinetic model. The cumulative exposure to the
449 four pyrethroids was estimated for each individual together with the cumulative risk. Our results
450 indicated that no risks for three neurotoxicity endpoints associated with reconstructed mixtures of
451 pyrethroids were expected for the ENNS cohort.

452 To our best knowledge, this study is the first to estimate the external exposure to mixtures of
453 pyrethroids using biomonitoring data. Some studies have either considered only one compound
454 (Tornero-Velez *et al.*, 2012; Wei *et al.*, 2013) or have applied bottom-up approaches, *i.e.* starting from
455 the contamination of the environmental media and food (Aylward *et al.*, 2018). In France, that latter
456 approach was applied to derive daily intakes for the four compounds (Vanacker *et al.*, 2020) or
457 permethrin only (Hermant *et al.*, 2017; Darney *et al.*, 2018). The comparison of the estimates of the
458 daily intakes obtained by bottom-up approaches and our top-down approach (*i.e.*, from the human
459 biomarkers) leads to similar conclusions. Both Vanacker *et al.* (2020) and Darney *et al.* (2018) came
460 up with three exposure scenarios based on the treatment of censored values in environment and food
461 contamination data. All our pyrethroids' intakes are within the range of their estimates for the
462 optimistic (or lower) and refined (or intermediate) scenarios. We also compared our estimates with
463 intakes from other countries. Recently, Aylward *et al.* (2018) calculated the dietary intakes of several
464 pyrethroids including permethrin, cypermethrin and deltamethrin for a US population, aged 6–79 years
465 old. The intakes' values highly differed between the two countries, as well as the contributions of the
466 pyrethroids to the cumulative exposure. Indeed, the US population is exposed mainly to permethrin
467 (1,800 ng/kg bw/day), then cypermethrin (610 ng/kg bw/day) and deltamethrin (1.9 ng/kg bw/day).
468 Similar rankings were also obtained for US children in another study (Xue *et al.*, 2014). Based on our
469 results, the ranking of pyrethroids exposure would be reversed for the French general population.
470 These differences between countries could be explained by the variety of agricultural practices related

471 to different types of crops, climate, dietary habits and other individual behaviours, such as the indoor
472 use of pesticides. Indeed, permethrin is one of the most used pyrethroid compounds in the US while it
473 is banned in France for agricultural use. Another explanation lies in the fact that only adults were
474 included in the French ENNS cohort, whereas the US study covers a population aged between 6 to 79
475 years old. Aylward *et al.* (2018) then considered exposures specific to children, as the non-dietary
476 contact which can significantly increase exposure of this population. These differences in pyrethroids'
477 exposures then prevent the use of the second tier BE value for 3-PBA based on the US exposure
478 estimates (Aylward *et al.*, 2018) for the French population. Our approach is then an alternative to their
479 method and enabled estimating the French exposure to mixtures of the four pyrethroids.

480 Accounting for inter-individual variability in reverse dosimetry from biomonitoring data is critical to
481 describe the natural variability of the population included in those data. In our approach, human inter-
482 individual variability was accounted by the means of probability distributions assigned to the
483 toxicokinetic parameters that most impact the urinary concentrations of the metabolites, *i.e.* the part of
484 a parent compound transformed into a metabolite (Quindroit *et al.*, 2019). In addition to the MCMC
485 simulations reported above, a deterministic simulation using the average parameter values was
486 performed for one individual in order to assess the impact of the metabolic parameters' variability on
487 the estimated dietary intakes. These simulations (not shown) indicated higher coefficients of variation
488 in the MCMC results for the all the parameters (from a 2- to a 4-factor) and higher 95th percentiles
489 than in the deterministic results. This comparison highlights the importance of associating the dietary
490 intakes with an interval representative of human variability. Neglecting that variability could lead to
491 underestimate the dietary intakes, and then to underestimate the risk associated to these exposures.

492 Regarding the posterior distributions of the metabolic parameters obtained from the MCMC
493 simulations, our results showed that the adjustment of the global model to the biomonitoring data
494 required to update some parameters at the individual or population level. At the population level, the
495 deviations between the prior and posterior distributions of the metabolic parameters point out the
496 discrepancies between the information on the metabolic activity implemented in the model and the
497 information contained in the biomonitoring data. Indeed, the PBPK models were parametrized with

498 observations from toxicokinetic studies including few human volunteers under controlled exposures.
499 Our simulations showed that only two metabolic parameters were affected by those deviations for all
500 the cohort: the transformation of deltamethrin into DBCA and *trans*-permethrin into 3-PBA. However,
501 the impact on the median is rather limited as the posterior medians did not deviate by more than 15%
502 from the prior median, and despite that decrease the values of these two metabolic fractions were still
503 the highest ones. At the individual level, these deviations can be explained by natural variability. In
504 those studies, more DCCA (about twice) was generated by the *trans* isomer than the *cis* one from
505 cypermethrin, permethrin and cyfluthrin (Woollen *et al.*, 1992; Leng *et al.*, 1997b; Ratelle *et al.*,
506 2015a; Ratelle *et al.*, 2015b). Regarding the isomeric *cis:trans* ratios (40:60 for permethrin and
507 cyfluthrin, and 42:58 for cypermethrin), it is then expected that each person has higher urinary levels
508 of *trans*-DCCA (about a 2-factor) than *cis*-DCCA ones. In most of the ENNS cohort, that factor was
509 found when both compounds were quantified (2.7 on average and interquartile interval [1.9; 3.1]).
510 However, for some individuals exhibiting ratios of *trans*- and *cis*-DCCA concentrations far from this
511 2-factor (either very low (between 0.4 and 0.9) or very high (6.3 and 18.5)), the distributions of
512 metabolic parameters were updated (up to 7 parameters) to fit the measured concentrations resulting in
513 posterior distributions deviating from the prior distributions. Overall, the posterior distributions of the
514 metabolic fractions were close to the prior ones, indicating a good agreement between the
515 toxicokinetic studies and the biomonitoring data.

516 This good agreement was obtained under the hypothesis of an additional 3-PBA production by other
517 pyrethroids that were not included in the model. Indeed, 3-PBA can be formed by many pyrethroids,
518 including cyhalothrin, phenothrin or esfenvalerate (Ueyama *et al.*, 2010). To our best knowledge, the
519 exposure to other pyrethroids than the four of interest has not been studied in France, as they are not
520 commonly included in monitoring studies (environment or food). It was then not possible to compare
521 our estimates of the additional 3-PBA production to any data. During the estimation process, that
522 additional production allowed some degrees of freedom to the posterior distributions of the metabolic
523 fractions to stick to the prior distributions even though the urinary concentrations of 3-PBA and of the
524 other metabolites did not fulfil the expected correlations observed in the controlled laboratory

525 experiments, *e.g.* for the metabolites (DCCA and 3-PBA) formed by the same parent compound. We
526 observed in our results that the higher the urinary concentration of *cis*- and *trans*-DCCA and 3-PBA,
527 the lower the proportion of 3-PBA from other sources. This proportion then influenced mainly the
528 model's goodness of fit at low concentrations and might have led to underestimate the exposure to the
529 four pyrethroids of interest. However, this underestimation is likely to only affect individuals
530 exhibiting low concentrations of the metabolites, and therefore exposed to low pyrethroids' levels.

531 Human biomonitoring data are usually composed by a non-negligible number of non-quantified or
532 non-detected samples. This does not necessarily mean that the compound of interest is not present, but
533 that the concentration is below the level that can be reliably quantified or detected by the analytical
534 method used. Several strategies are possible for processing with this censored data. Simple methods
535 set the non-quantified data to 0, half of the limit of detection/quantification of the analytical methods,
536 or equal to this limit (EPA, 2000; EFSA, 2012). Depending on the methodology used, there is
537 potentially a risk of underestimating the actual exposure. In our study, a statistical model was defined
538 to allow the non-quantified samples to vary between the limit of quantification and zero. Our results
539 showed that this method enables estimating a non-quantified concentration of one metabolite in
540 accordance with the quantified concentrations of the other metabolites. Compared to the simple
541 methods, our model is more likely to be close to the actual concentration, even though for some
542 metabolites it has been shown that no information was contained in the other metabolites'
543 concentrations to inform the concentration of the unquantified metabolite (*e.g.*, DBCA that is specific
544 to deltamethrin).

545 Several difficulties arose during the estimation process of the daily intakes from the biomonitoring
546 data. The main one was that cypermethrin and permethrin share the same metabolites among the five
547 measured in the cohort. It then would not have been possible to distinguish the part of the urinary
548 concentrations of the metabolites DCCA and 3-PBA formed by one or the other of these compounds,
549 and that would lead to the nonuniqueness of the intakes of cypermethrin-permethrin pair (*i.e.*, an
550 infinity of solutions). To overcome that, a realistic weighted exposure ratio between permethrin and
551 cypermethrin was calculated using the food diaries of the ENNS participants and food contamination

552 data for France. Even though that approach is subject to uncertainties in the reporting and in the
553 contamination data that were very sparse for pyrethroids (considering that most of the food samples
554 were below the limit of quantification), an exposure ratio between these two compounds was
555 calculated for each participant. The permethrin daily intakes were therefore not directly estimated but
556 was obtained by multiplying this ratio and the cypermethrin intake's estimate. An alternative would
557 have been to maximize the risk by considering only the most toxic compound between these two, *i.e.*
558 cypermethrin (Table 4), during the estimation process. That would lead to probably an overestimation
559 of the exposure for cypermethrin and the associated risk. Our results showed that the ENNS cohort
560 seemed slightly more exposed to cypermethrin than permethrin, that is consistent with real-world
561 exposure since European regulations classified permethrin as an unapproved active substance for
562 agricultural use in 2000. This is also consistent with the intakes estimated for all the routes of exposure
563 including dermal contact and inhalation, by Vanacker *et al.* (2020) that indicated a higher exposure to
564 cypermethrin than to permethrin by a 2-factor at least (for low and moderate exposures).

565 The estimated exposures and the associated risks rely on assumptions about the exposure scenario and
566 the sampling of the urinary concentrations. Only the ingestion route was modelled in our exposure
567 scenario even if the global model integrates also inhalation and dermal contact (Quindroit *et al.*, 2019).
568 Our choice was supported by previous studies for the French population that showed that for almost all
569 the population (99%) diet was the main contributor to the aggregated pyrethroids' exposure, and that
570 for the remaining 1% veterinary and medicine products were important sources leading possibly to
571 very high levels of permethrin intakes (Darney *et al.*, 2018; Vanacker *et al.*, 2020). Regarding the
572 ENNS cohort, no participant showed very high levels of the urinary concentrations of the permethrin's
573 metabolites (*cis*- and *trans*-DCCA, and 3-PBA) suggesting that this second source of exposure was
574 also minor for the ENNS cohort.

575 Our scenario was also constrained by the sampling of the biomonitoring data. In the ENNS cohort, the
576 first morning voids were collected. The reliability of single-spot urine to be representative of the
577 pyrethroid exposure over time is questionable due to their rapid elimination from the body (Clewell *et al.*,
578 *et al.*, 2008). The variability of spot samples of pyrethroids has been discussed in the literature but no

579 consensus was reached on its impact on exposure classification (Wielgomas, 2013; Morgan *et al.*,
580 2016; Li *et al.*, 2019). For instance, the studies by Li *et al.* (2019) and Morgan *et al.* (2016) showed a
581 high within-individual variability of the 3-PBA concentration in spot and first morning voids samples
582 and concluded that such samples do not provide a reliable estimate of the average 3-PBA
583 concentration over a day. They also found that a very high number of spot urine samples (15–800 for
584 Li *et al.* (2019) and 18-140 for Morgan *et al.* (2016)) would be required per person to provide a
585 reliable concentration estimate over a day. These results are in contrast to Wielgomas (2013) that
586 observed a low within-individual variability in 3-PBA concentrations in spot and first morning voids
587 samples of Polish adults, and concluded that a random spot urine sample would adequately represent
588 the average 3-PBA biomarker concentration for an individual over a week. Nevertheless, other studies
589 for non-persistent compounds as pyrethroids tend to support a high within-individual variability in
590 spot and first morning voids samples (Aylward *et al.*, 2017; LaKind *et al.*, 2019). In new
591 biomonitoring studies, the sampling protocol for non-persistent compounds takes into account these
592 considerations in order to provide robust exposure estimates (Vrijheid *et al.*, 2014). Nevertheless,
593 numerous HBM studies have relied in the past on spot sampling leading to valuable data on human
594 exposure, even though affected by uncertainties. In that context, the study performed by Aylward *et al.*
595 (2017) with non-persistent compounds other than pyrethroids helps in characterizing the uncertainties
596 of exposure assessment when using spot samples. They showed that the use of a 95th percentile from
597 the distribution of population spot sample concentrations will provide a reasonable (conservative)
598 estimate of the 95th percentile of the distribution of 24 h average concentrations. Therefore, reverse
599 dosimetry using spot samples can provide a reasonable estimate of the upper tail of the exposures’
600 distribution.

601 The final step of our approach was to use the exposure estimates to the mixtures of the four
602 pyrethroids to compute the cumulative risks for functional alterations of the motor division, the
603 sensory division and the autonomic division. Our method was based on the computation of MOEs for
604 each individual using NOAELs from the three specific effects established by EFSA (2019a). All
605 individual MOEs were higher than 100 indicating no risks for these effects on the nervous system in

606 the French ENNS cohort. Similar results have been observed in other studies that computed the risks
607 not from HBM data as done here, but from the contamination of food and environment, and the
608 consumption of the populations (Li *et al.*, 2016; Vanacker *et al.*, 2020). As pointed out by EFSA
609 (2019a), the methodology is affected by some uncertainties and limitations in the available data and
610 scientific knowledge. For instance, no dedicated studies were available to estimate the cyfluthrin's
611 NOAEL for the functional alterations of the sensory and autonomic division. A pharmacology study
612 was then used as surrogate, leading to low NOAEL values compared to the other pyrethroids (Table
613 4).

614 Our results are of course limited to the exposure of the four pyrethroids of interest in this study, but
615 they also showed that for some individuals the proportion of 3-PBA produced by other sources than
616 the four pyrethroids can be significant (the median value is 36%). It could therefore be envisaged to
617 extend our approach to other pyrethroids that are metabolized into 3-PBA (*e.g.*, cyhalothrin or
618 esfenvalerate) and that are present on the EU market. This would provide a more accurate picture of
619 exposure to this chemical family and refine of the cumulative risk assessment associated to their
620 neurotoxic potential. The extension would imply to parametrize the PBPK model for the new
621 pyrethroid and one-compartment models for its metabolites. Nevertheless, the pyrethroids'
622 concentrations measured in the current HBM studies do not included the second metabolite of those
623 compounds (such as the chrysanthemumdicarboxylic acid) and that could render uncertain the
624 estimation of the exposure to these new pyrethroids. Another possible extension of our work would be
625 to consider other pesticides such as the ones identified by EFSA in the three CAGs on the functional
626 alterations of motor, sensory and automatic division (EFSA, 2019a). Indeed, HBM studies, including
627 the ENNS study (Fréry *et al.*, 2011), have shown that humans are exposed to a multitude of pesticides
628 in real life. PBPK models are available for several of those pesticides and could be used, in the same
629 way as done in this work, to estimate the human exposures from the HBM data. These estimated
630 exposures could then be included in the risk assessment in order to provide risk estimates relevant for
631 mixtures of pesticides known to alter the motor, sensory or autonomic functions. Another possible
632 extension of our work would be to account for other population groups like pregnant women and their

633 fetuses or children that are a sensitive population for pyrethroids in terms of exposure (Schulz *et al.*,
634 2009; Barr *et al.*, 2010; Egeghy *et al.*, 2011) and effects (Shafer *et al.*, 2005; Egeghy *et al.*, 2011). The
635 PBPK model could be easily adapted to children as it already integrates the physiological and
636 anatomical changes due to age (Beaudouin *et al.*, 2010) and that the toxicokinetics of pyrethroids in
637 children have already been characterized (Mallick *et al.*, 2020).

638 **5 Conclusions**

639 In this article, we presented a method for interpreting HBM data in the context of the exposure and
640 risk assessment continuum by the means of PBPK modelling. To our knowledge, this is the first time
641 that reverse dosimetry using PBPK modelling has been used to estimate the cumulative exposure to
642 pyrethroids' mixtures from their urinary metabolites' concentrations. Nowadays, many countries have
643 established biomonitoring programs to assess the chemical exposure in populations which allows the
644 building of large databases gathering biomarkers of exposure and individual information (Alimonti *et*
645 *al.*, 2011; Fréry *et al.*, 2011; Centers for Disease Control and Prevention, 2019; Health Canada, 2019).
646 Several initiatives, like the HBM4EU project (www.hbm4eu.eu/) have been carried out at national and
647 European levels to encourage the development of new technologies, tools and models to reduce the
648 uncertainties in exposure assessment from such data. In that context, PBPK modelling has been
649 acknowledged to aid the interpretation of these data by accounting for the behaviour of the compounds
650 in the body and the physiology of the individual, thus explaining some of the variability observed in
651 the biomarkers of exposure (Clewell *et al.*, 2008; Sarigiannis *et al.*, 2019a). Our approach is then an
652 asset to analyse the biomarkers of exposure to pyrethroids. Because our approach only necessitates
653 HBM data and exposure scenarios relevant for the population of interest, it could capture any local or
654 national specificities in terms of pyrethroids' exposure.

655 **Conflict of interest declaration**

656 The authors declare that they have no conflict of interest.

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661

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