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Highlights

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

Abstract

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

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

1 Introduction

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

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

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

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

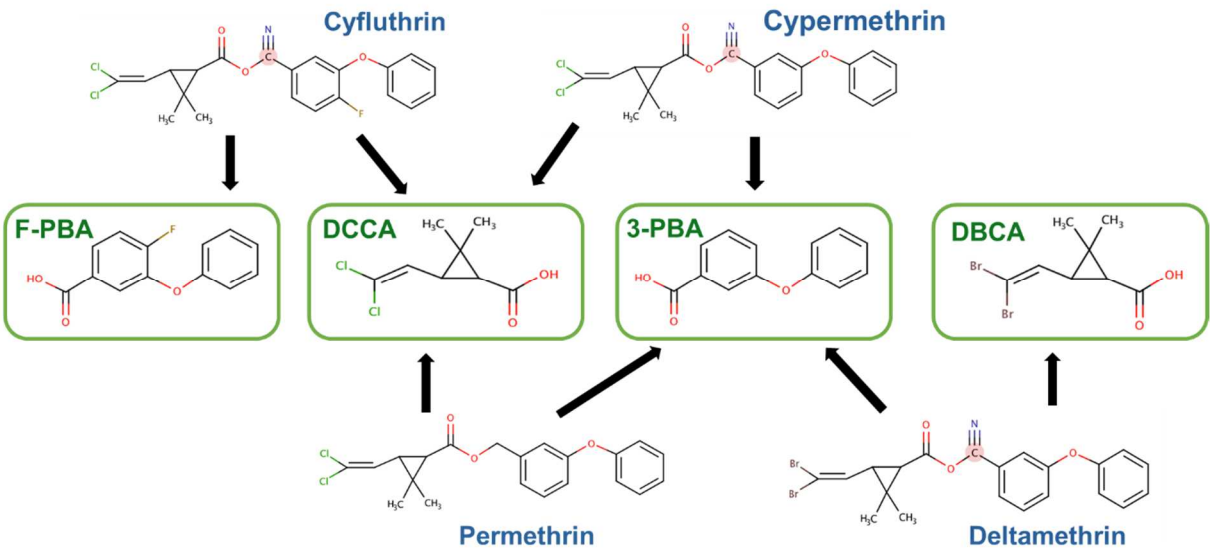

Figure 1: Pyrethroids and their urinary metabolites measured in biomonitoring studies, adapted from Ueyama et al. (2010) and Starr et al. (2008)

2 Materials and Methods

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

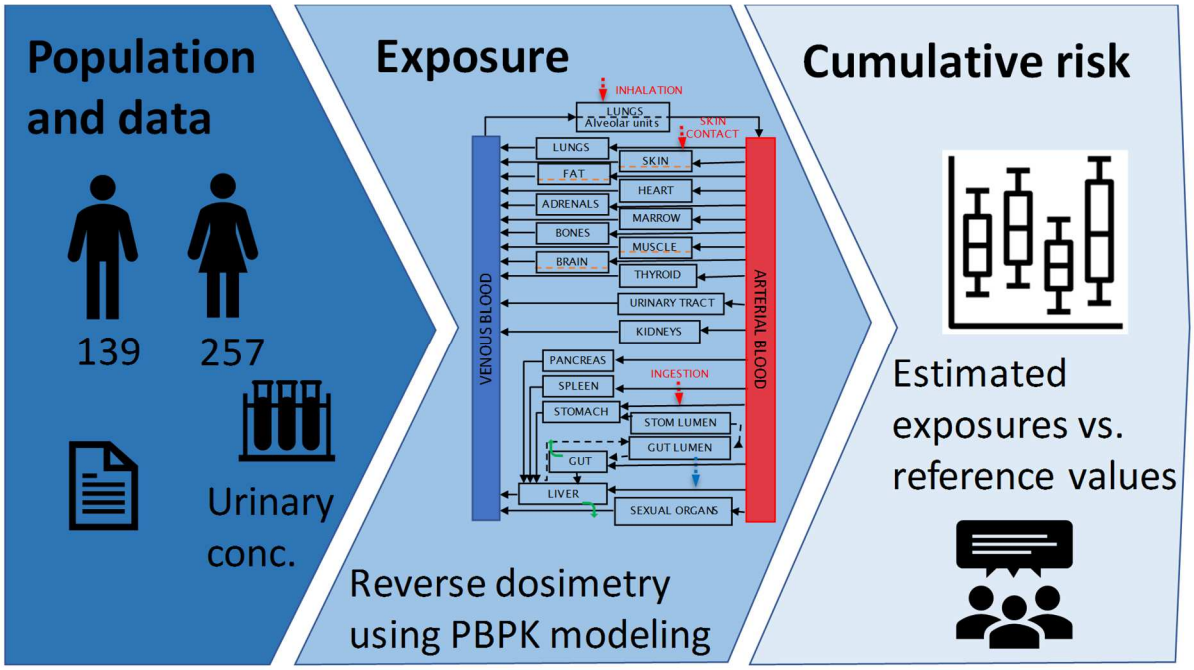

Figure 2: Workflow of our approach to estimate the exposure and the cumulative risk to pyrethroids for the ENNS cohort.

2.1 Study population

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

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

2.2 Chemicals and biomonitoring data

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

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

Table 1: Validation criteria of the analytical method used for measuring the pyrethroids' 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

Table 2: Summary of individual characteristics and the urinary concentrations of the pyrethroids' metabolites (µg/L) for the ENNS participants (n total = 396, n women = 257, n men = 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)		
cis-DCCA	0.13±0.53 [<LOQ; 1.43]	0.14±1.48 [<LOQ; 3.32]
trans-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 (%)		
cis-DCCA	55	57
trans-DCCA	88	85
DBCA	89	80
F-PBA	31	29
3-PBA	99	98

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

2.3 Assessment of the exposures to pyrethroids

2.3.1 PBPK model for pyrethroids

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

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

2.3.2 Exposure scenario

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

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

To reconstruct the pyrethroids' exposures from the HBM data, we considered a continuous exposure *via* oral intake for each parent compound in order to reach a steady-state. The daily intakes for 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 $$P(DI|U) = \frac{P(U|DI)}{\sum[P(U|DI_i)]}$$

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

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

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

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

Fraction of metabolites	Distributions	Truncation
trans-Permethrin		
trans-DCCA	Normal(0.61,0.18)	[0.12-1.00]
3-PBA	Normal(0.85,0.26)	[0.17-1.00]
cis-Permethrin		
cis-DCCA	Normal(0.37,0.11)	[0.07-1.00]
3-PBA	Normal(0.37,0.11)	[0.07-1.00]
trans-cypermethrin		
trans-DCCA	Normal(0.57,0.17)	[0.11-1.00]
3-PBA	Normal(0.39,0.12)	[0.08-1.00]
cis-Cypermethrin		
cis -DCCA	Normal(0.32,0.10)	[0.06-1.00]
3-PBA	Normal(0.16,0.05)	[0.03-1.00]
trans-Cyfluthrin		
trans-DCCA	Normal(0.35,0.11)	[0.07-1.00]
F-PBA	Normal(0.23,0.07)	[0.05-1.00]
cis-Cyfluthrin		
cis-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]

2.4 Cumulative risk assessment

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

$$E_{cumul} = \sum_{x} (DI_x \times RPF_x)$$

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

$$MOE = NOAEL_{DLT} / E_{cumul}$$

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

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

Table 4: NOAELs and the corresponding toxicological endpoints of the four pyrethroids for the EFSA CAGs on functional alterations of the motor division, the sensory division and the autonomic division to be used in chronic exposure/risk assessments (EFSA, 2019a). Deltamethrin 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

3 Results

3.1 Statistical analyses of the biomonitoring data

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

3.2 Ratio of exposure between permethrin and cypermethrin

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

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

3.3 Estimation of the daily intakes from the urinary concentrations

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

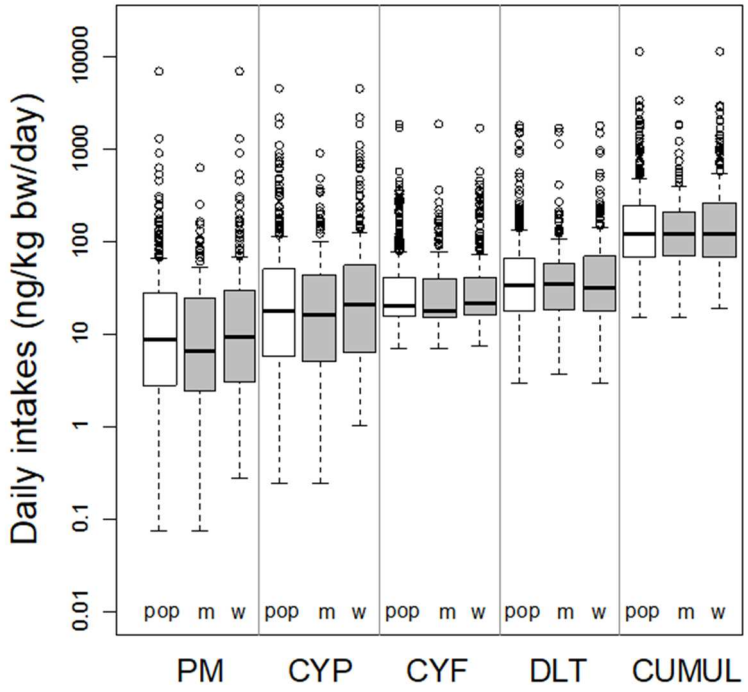

Figure 3: Mean daily intakes for permethrin (PM), cypermethrin (CYP), cyfluthrin (CYF), deltamethrin (DLT) and the cumulative exposure (CUMUL) in the whole population (*pop*), men (*m*) and women (*w*) estimated from the urinary metabolites' concentrations using the global model. The first quartile,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

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

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

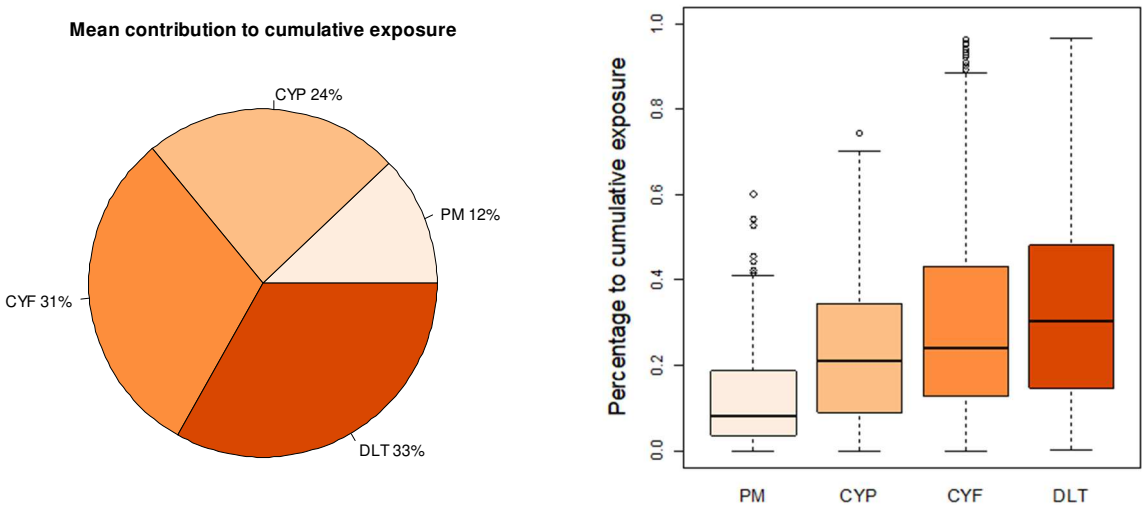

Figure 4: Mean contribution (left) and distributions of the contributions (right) of each parent compound to the pyrethroids' cumulative exposure in the ENNS cohort.

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

3.4 Estimation of the individual parameters

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

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

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

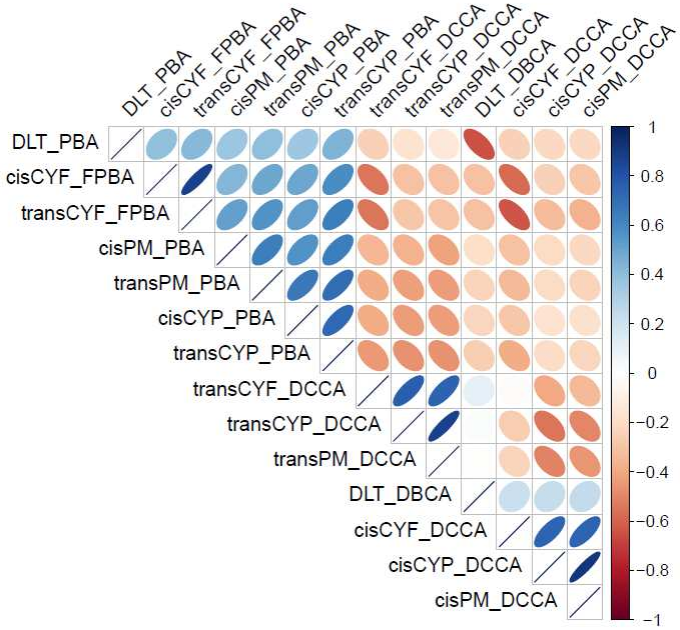

Figure 5: Spearman correlations between the 14 metabolic fractions at the population level. The individual means of the metabolic fraction estimated were used.

3.5 Cumulative risk assessment

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

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

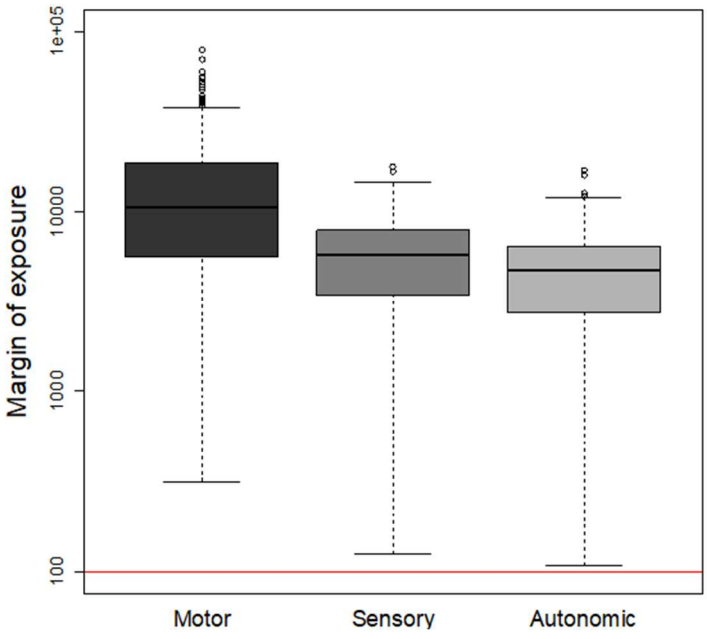

Figure 6: Distribution of the 5th percentile of the individual distribution of the margin of exposure for three neurotoxic effects

4 Discussion

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

To our best knowledge, this study is the first to estimate the external exposure to mixtures of pyrethroids using biomonitoring data. Some studies have either considered only one compound (Tornero-Velez *et al.*, 2012; Wei *et al.*, 2013) or have applied bottom-up approaches, *i.e.* starting from the contamination of the environmental media and food (Aylward *et al.*, 2018). In France, that latter approach was applied to derive daily intakes for the four compounds (Vanacker *et al.*, 2020) or permethrin only (Hermant *et al.*, 2017; Darney *et al.*, 2018). The comparison of the estimates of the daily intakes obtained by bottom-up approaches and our top-down approach (*i.e.*, from the human biomarkers) leads to similar conclusions. Both Vanacker *et al.* (2020) and Darney *et al.* (2018) came up with three exposure scenarios based on the treatment of censored values in environment and food contamination data. All our pyrethroids' intakes are within the range of their estimates for the optimistic (or lower) and refined (or intermediate) scenarios. We also compared our estimates with intakes from other countries. Recently, Aylward *et al.* (2018) calculated the dietary intakes of several pyrethroids including permethrin, cypermethrin and deltamethrin for a US population, aged 6–79 years old. The intakes' values highly differed between the two countries, as well as the contributions of the pyrethroids to the cumulative exposure. Indeed, the US population is exposed mainly to permethrin (1,800 ng/kg bw/day), then cypermethrin (610 ng/kg bw/day) and deltamethrin (1.9 ng/kg bw/day). Similar rankings were also obtained for US children in another study (Xue *et al.*, 2014). Based on our results, the ranking of pyrethroids exposure would be reversed for the French general population. 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 95^{th} 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

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

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

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

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

Several difficulties arose during the estimation process of the daily intakes from the biomonitoring data. The main one was that cypermethrin and permethrin share the same metabolites among the five measured in the cohort. It then would not have been possible to distinguish the part of the urinary concentrations of the metabolites DCCA and 3-PBA formed by one or the other of these compounds, and that would lead to the nonuniqueness of the intakes of cypermethrin-permethrin pair (*i.e.*, an infinity of solutions). To overcome that, a realistic weighted exposure ratio between permethrin and 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*
578 *al.*, 2008). The variability of spot samples of pyrethroids has been discussed in the literature but no

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

The final step of our approach was to use the exposure estimates to the mixtures of the four pyrethroids to compute the cumulative risks for functional alterations of the motor division, the sensory division and the autonomic division. Our method was based on the computation of MOEs for each individual using NOAELs from the three specific effects established by EFSA (2019a). All 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

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

5 Conclusions

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

Conflict of interest declaration

The authors declare that they have no conflict of interest.

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