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Prenatal exposure to chlorpyrifos of French children from the Elfe cohort

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ABSTRACT

Background: The organophosphate pesticide chlorpyrifos was widely used in the European Union before its ban in 2020 and was associated with neurodevelopmental disorders. However, within the concept of Developmental Origins of Health and Disease, *in utero* exposure to chlorpyrifos can lead to neurodevelopmental effects in developing children.**Objective:** The aim of this study was to estimate fetal exposure to chlorpyrifos using biomonitoring data measured in Elfe pregnant women and a physiologically based pharmacokinetic (PBPK) approach and compare exposure to toxicological reference values.**Methods:** A pregnancy-PBPK model was developed based on an existing adult chlorpyrifos model and a new toxicological reference value was proposed for neurodevelopmental effects. The pregnant women exposure was estimated based on dialkylphosphate (DAP) levels in urine assuming constant exposure to chlorpyrifos and compared to both the existing toxicological reference value and the new proposed draft toxicological reference value. Fetal internal concentrations in target tissues were then predicted using the developed pregnancy-PBPK model. Urinary concentrations of the chlorpyrifos-specific metabolite (TCPy) were also predicted for comparison with other biomonitoring data.**Results:** The median daily exposure to chlorpyrifos for the French pregnant women from the Elfe cohort was estimated at 6.3×10^{-4} $\mu\text{g}/\text{kg}$ body weight/day. The predicted urinary excretion of TCPy, the chlorpyrifos-specific metabolite, is in the same range as observed in other European cohorts (mean: 2.13 $\mu\text{g}/\text{L}$). Predicted brain chlorpyrifos levels were similar in pregnant women and their fetus and were 10-fold higher than the predicted blood chlorpyrifos levels. It was estimated that 6% and 20% of the pregnant women population had been exposed to levels exceeding the general population and draft toxicological reference values, respectively.**Conclusions:** Prenatal exposure to chlorpyrifos was estimated for the French population based on data from the Elfe cohort. Internal chlorpyrifos concentrations in target tissues (brain and blood) were predicted for fetuses at the end of the pregnancy. Under a conservative assumption, a small percentage of the population was identified as being exposed to levels exceeding the toxicological reference values.

Abbreviations: BBB, blood-brain barrier; BNVD, French National Bank of Distributor Sales; CPF, chlorpyrifos; CPF-oxon, chlorpyrifos-oxon; CYP 450, cytochrome P450; DAPs, dialkylphosphates; DEP, diethylphosphate; DETP, diethylthiophosphate; DOHaD, developmental origins of health and diseases; EU, European union; HBM, human biomonitoring; Ined, National Institute of Demographic Studies; Inserm, National Institute of Health and Medical Research; LOAEL, low observed adverse effect level; LOD, limit of detection; LOQ, limit of quantification; MCMC, Markov Chain Monte Carlo; OCLN, occludin; PON1, A-esterase; p-PBPK, pregnancy physiologically based pharmacokinetics; TCPy, 3,5,6-trichloro-2-pyridinol; TRV, toxicological reference value.

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1. Introduction

Chlorpyrifos (CPF), an organophosphate pesticide, was widely applied as an insecticide in agriculture and as a biocide to control non-agricultural pests, until the implementation of regulatory measures such as restrictions or bans as in Europe in 2020. In fact, it was the most widely used pesticide in food production in the European Union (EU) until 2020 (Wolejko et al., 2022). Several studies have reported correlations between prenatal or childhood exposure to CPF and neurodevelopment disorders observed in humans at various life stages such as learning disabilities, reduced intellectual quotient, attention deficit and hyperactivity disorders (Guo et al., 2019; Rauh et al., 2011; Wei et al., 2023). There is a growing concern about the delayed effects of prenatal exposure to pesticides on children's health. According to the concept of Developmental Origins of Health and Disease (DOHAD) (Barker, 1998; Rager et al., 2020), prenatal exposure to toxics including pesticides are potential risk factors for the onset of neurodevelopmental disorders in children. Several studies demonstrated that CPF can easily pass through the placenta and affect the fetus (Abdel-Rahman et al., 2002; Saulsbury et al., 2008; Whyatt et al., 2009). Maternal and neonatal exposures to many xenobiotics, including organophosphate pesticides, have been assessed in human biomonitoring (HBM) programs in France such as the Elfe cohort study (Etude Longitudinale Française de l'Enfance – French Longitudinal Study since Childhood) (Dereumeaux et al., 2016). Urinary levels of two CPF metabolites, diethylphosphate (DEP) and diethylthiophosphate (DETP), were measured in pregnant women in the Elfe cohort. These two dialkylphosphates (DAP) are biotransformation products of many organophosphate pesticides. Among these organophosphates, chlorpyrifos-ethyl was the main organophosphate pesticide sold in France at the time of the Elfe cohort sampling. CPF biotransformation leads also to urinary excretion of 3,5,6-trichloro-2-pyridinol (TCPy), which is specific to CPF and is commonly used to monitor CPF exposure in HBM studies (Fig. 1).

Several methods can be used to estimate the exposure to xenobiotics based on biomonitoring data, including steady-state equations (Tarazona et al., 2022; Bartels et al., 2012) and physiologically based pharmacokinetics (PBPK) modeling. The steady-state equation, which can also be called a mass balance equation, assumes a linear relationship between the excretion rates of major metabolites and ingestion rates under steady-state conditions. Biomarker measurements in urine are therefore translated into exposure estimates.

In order to relate exposure and HBM data to internal maternal and fetal concentrations, PBPK modeling can be used (Ali Daoud et al., 2023; Brochot et al., 2019; Ratier et al., 2024). PBPK models describe the

absorption, distribution, metabolism and excretion of a substance in the organism. Several PBPK models for chlorpyrifos have been previously developed for the adult non-pregnant population (Lowe et al., 2009; Timchalk et al., 2002; Zhao et al., 2019). To our knowledge, there is currently no pregnancy-PBPK (p-PBPK) model that takes into account chlorpyrifos metabolite excretion. In this work, a lifetime model including pregnancy specific to CPF and including the urinary excretion of both DAPs and TCPy was developed. PBPK models are useful for linking dietary intake estimated by the steady-state equation to the internal concentrations over time. Prediction of internal concentrations in fetuses can provide information on fetal kinetics during critical periods of development, if identified, and can therefore be used to link internal concentrations and effects.

External exposure estimates to CPF can also be considered in a risk assessment context. Several toxicological reference values (TRVs) were established by different agencies and organizations to assess the risk associated with exposure to CPF (ATSDR, 1997, EFSA, 2014, HEALTH CANADA, 2003; NHMRC, 2011, OEHHA, 2010, WHO, 2022, WHO JMPR, 1999). These TRVs have been established for acute, sub-chronic and chronic exposure to CPF for the general population. In this study, a new value derived from a study highlighting neurodevelopmental toxicity in pups following exposure to chlorpyrifos in pregnant rats (referred to as the draft toxicological reference value hereafter) was proposed.

The aims of this study were i) to estimate the dietary intake of chlorpyrifos based on urinary metabolite (dialkylphosphates) concentrations for pregnant women from the Elfe cohort assuming steady-state conditions, ii) to estimate TCPy urinary concentrations for pregnant women and to predict the internal concentration for fetuses at the end of the pregnancy using the developed p-PBPK model, and iii) to compare the pregnant population exposure levels with the threshold recommended to prevent risks for children health (TRV).

2. Methods

2.1. Workflow from prenatal exposure to risk assessment

The following study can be presented in a workflow describing briefly its different steps (Fig. 2). First, exposure to CPF in the Elfe pregnant women was estimated using a steady-state equation. In a risk assessment context, the exposure estimates to CPF were compared to existing TRV for the general population and also to a draft toxicological value specific to neurodevelopment derived from *in vivo* experiments conducted in pregnant rats.

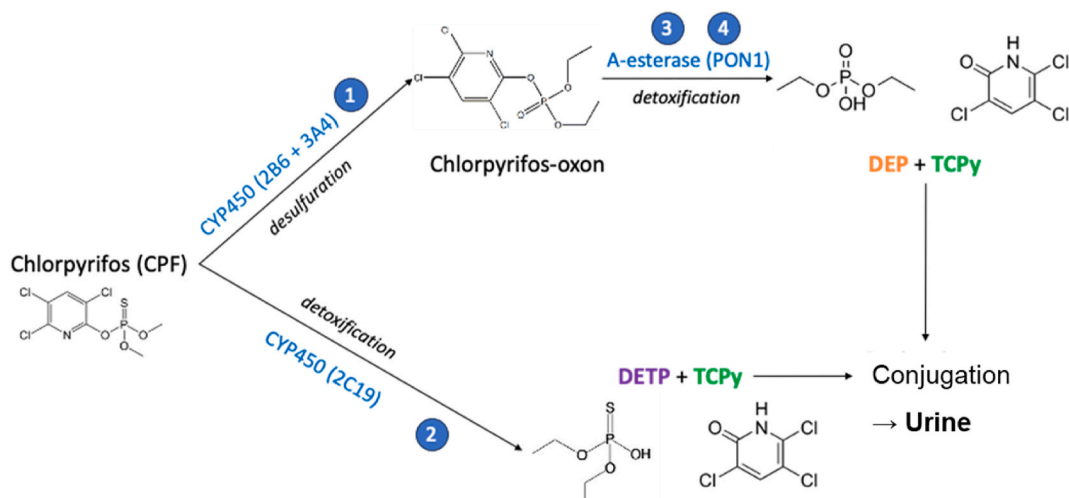


Fig. 1. Representation of CPF biotransformation in TCPy, DEP and DETP (adapted from Zhao et al. (2022)).

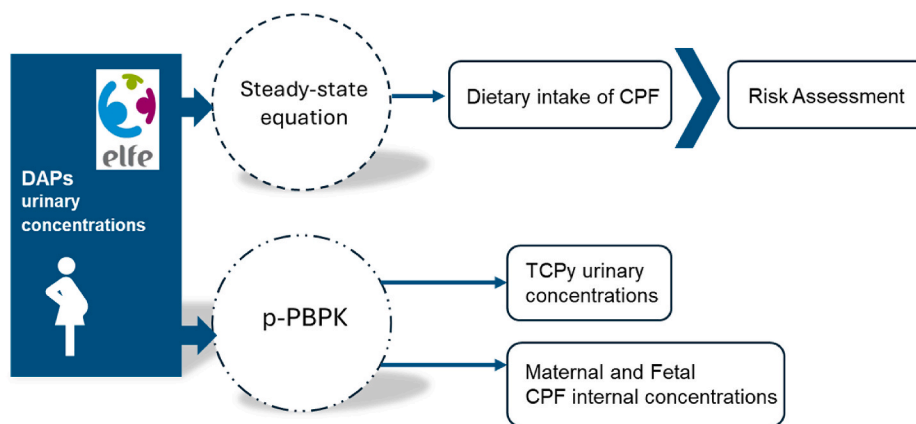


Fig. 2. Workflow of this study: from DAPs urinary concentration to risk assessment. p-PBPK: pregnancy physiologically based pharmacokinetics; DAPs: dialkylphosphates; TCPy: 3,5,6-trichloro-2-pyridinol; CPF: chlorpyrifos.

TCPy urinary concentration and internal concentrations in pregnant women and fetuses were predicted using the p-PBPK model developed in this study.

In this work, since a steady state was assumed, the use of a steady-state equation or the PBPK led to the same estimated daily intake of CPF. Therefore, we chose to present in this work, as a first step, the steady-state equation to estimate the exposure of the Elfe population to CPF.

2.2. Study population

$$FEED = \left(DAP_{UrinceConc}^* \text{ Daily urine volume adjusted to bw}^* \frac{MW_{Chlorpyrifos}}{MW_{DAPs}^* F_{ue}} \right) / FA \quad \text{Equation 1}$$

The Elfe (Étude longitudinale depuis l'enfance) cohort is a French longitudinal cohort launched in 2011 by the National Institute of Demographic Studies (Ined) and the National Institute of Health and Medical Research (Inserm). The analysis of the samples and the dosages were part of the French HBM program. Over 18,000 children born in France in 2011 were enrolled in the Elfe cohort to characterize factors that may influence the child's physical or psychological development, health and socialization. Detailed information is available on the study website: <http://www.elfe-france.fr>. Biological samples were collected from pregnant women at delivery in a subset of the Elfe cohort (n = 899). The available data do not include urinary concentrations of DAPs from subjects who later discontinued the study.

Exposure of the pregnant Elfe population to xenobiotics was assessed by measuring urinary metabolite concentrations in samples collected at delivery (Dereumeaux et al., 2016). Only DAPs (DEP and DETP) were measured in pregnant women urine samples (Supplementary Material 1). Levels of both DAPs were above the detection limit in only a few maternal urine samples (Dereumeaux et al., 2016) (Table 1). DEP and DETP were detected in urine samples of 4.2% and 20.0% of the

Table 1

Urinary metabolite concentrations (P50: median, P95: 95th percentile, P99: 99th percentile) for pregnant women (n = 899) measured in the Elfe cohort ($\mu\text{g/L}$) and associated limits of detection (LOD) and limits of quantification (LOQ).

Urinary metabolite	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)	P50	P95	P99
DETP	0.2	0.6	<LOD	2.7	5.1
DEP	0.2	0.6	<LOD	<LOD	13.9

population, respectively.

2.3. Fractions excreted in urine as DEP and DETP

Based on the equation developed by Tarazona et al. (2022a), the dietary intake of chlorpyrifos was estimated for each of the 899 pregnant women. For low persistent compounds such as chlorpyrifos (half-life: 27h in blood (Hathaway and Proctor, 2004; ACGIH, 2012)) and assuming a steady state, the dietary intake can be estimated using a simple equation (Equation (1))(Bartels et al., 2012; Tarazona et al., 2022a).

Where *FEED* is the chlorpyrifos daily intake ($\mu\text{g/kg}$ body weight (bw)/day), is the measured DEP or DETP urinary concentration ($\mu\text{g/L}$), and are respectively the metabolite and chlorpyrifos molecular weight (g/mol), *F_{ue}* is the fraction of urinary excretion (%), *FA* is the absorbed fraction and the daily urine volume adjusted to body-weight (L/kg bw/day). DEP (MW: 154.10 g/mol) and DETP (MW: 170.17 g/mol) fractions of urinary excretion (*F_{ue}*) were based on the fractions of 87% and 13% of absorbed dose of CPF excreted as DEP and DETP respectively estimated by performing simulations with a constant continuous exposure to 145 $\mu\text{g/day/kg}$ bodyweight CPF with the PBPK model for chlorpyrifos described by Zhao et al. (2022). At this exposure level, saturation of metabolic processes was negligible and the model can therefore be considered to be linear. A daily urinary flow of 0.02 L/kg bw/day (Tarazona et al., 2022a) and an absorbed fraction (*FA*) of 0.462 were considered (Zhao et al., 2022).

2.4. Pregnancy-PBPK model

A PBPK model was developed to describe the kinetics of CPF and chlorpyrifos-oxon (CPF-oxon) in humans at all life stages, including pregnancy, after oral exposure. The structure is based on the adult model of Zhao et al. (2019), which was derived from the model developed by Timchalk et al. (2002). The model developed by Zhao et al. (2019) differs from model proposed by Timchalk et al. (2002)

mainly by including urinary excretion of DAPs and considering CPF metabolism occurs only in blood and liver, as the contributions of other organs (diaphragm and brain) in Timchalk et al.'s (2002) model

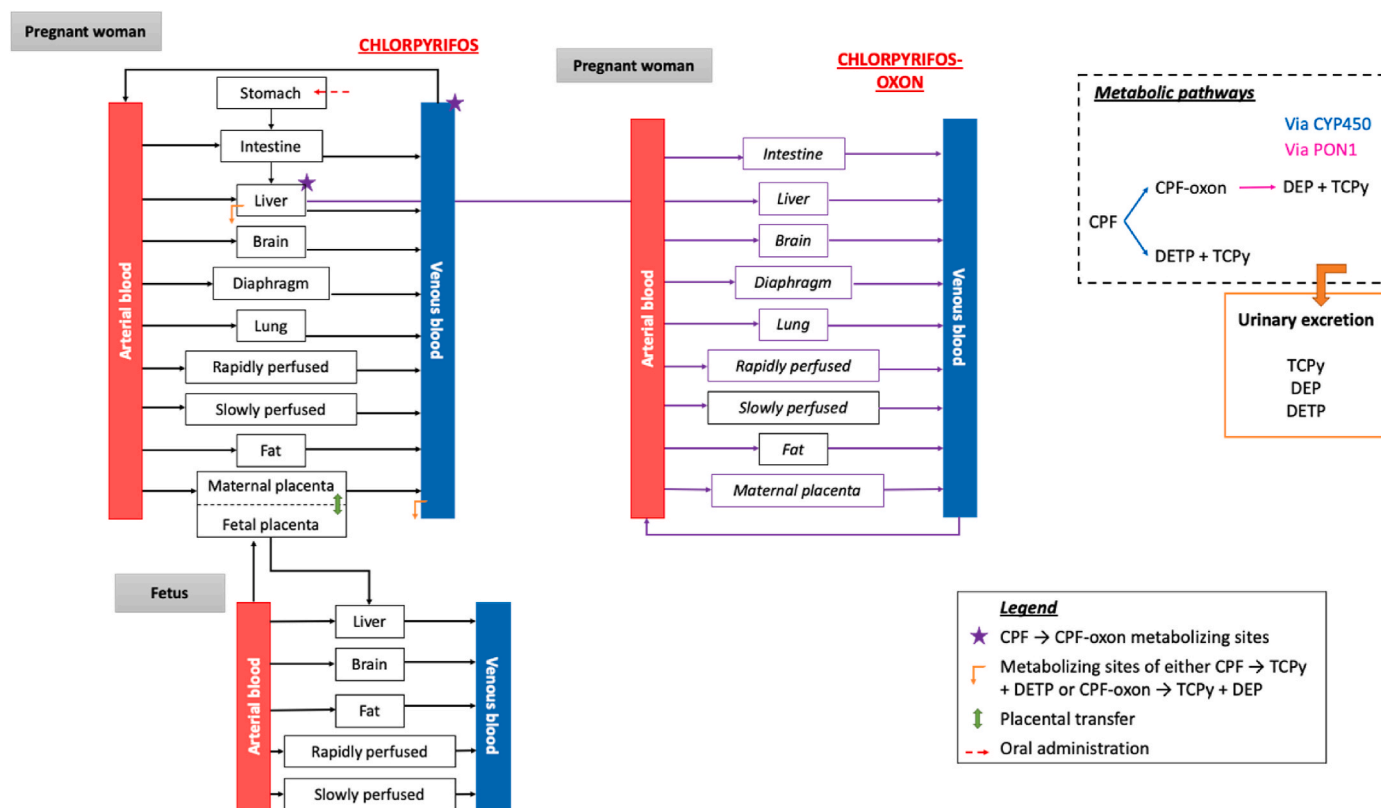


Fig. 3. Pregnancy-PBPK model schematic view.

are of minor importance.

In the present work, the model of Zhao et al. (2019) was extended to account for pregnancy by adding compartments for placenta and embryo/fetal tissues (Fig. 3). The embryo/fetal submodel consists of 6 compartments (blood, liver, brain, adipose tissue, rapidly and slowly perfused tissues) and is connected to the maternal part of the model (11 compartments) via the placenta, which is divided into maternal and fetal parts (Supplementary Material 2). As described by Ali Daoud et al. (2023), changes in maternal body weight, compartment volumes and blood flows during growth and pregnancy were considered in our model

Table 2
Substance specific parameters used in the p-PBPK model.

Parameter (unit)	Value	Reference
Tissue:blood partition coefficients		
	CPF	CPF-oxon
Adipose tissue	142	119.3
Liver	8.1	4.9
Slowly perfused	5.2	3.3
Rapidly perfused	8.1	4.9
Placenta	5.82	2.25
Metabolism		
	CYP2B6	CYP3A4
Vmax ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{kg of tissue}^{-1}$)	114.4	514.4
Km (μM)	0.14	29.77
Other parameters		
Absorbed fraction	0.462	Zhao et al. (2022)
TCPy elimination rate (h^{-1})	0.019	Zhao et al. (2019)
DAPs elimination rate (h^{-1})	0.199	Bouchard et al. (2005)
Constant of placental diffusion ($\text{L}/\text{hour}/\text{dm}^2$)	1	-
Distribution volume (L)	15	Zhao et al. (2019)

as a function of bodyweight and gestational age (Abduljalil et al., 2021; Dallmann et al., 2017; Zhang et al., 2017). Fetal organ growth (Beaudouin et al., 2010; ICRP, 2002; Luecke et al., 1994) and changes in blood flows (Abduljalil et al., 2021) were also included in the model. Fetal hematocrit evolution was described as in Dallmann et al. (2017).

Substance specific parameters used in the model are presented in Table 2. The maternal partition coefficients were set to the values from Zhao et al. (2019), for CPF and CPF-oxon. Brain, diaphragm, stomach, intestines and lung:blood partition coefficients were set to the same values as for rapidly perfused organs. Fetal partition coefficients were set to the same values as in the maternal submodel due to the lack of data on CPF and CPF-oxon distribution in the fetus (Thépaut et al., 2023). The placental partition coefficients of CPF and CPF-oxon were set to those estimated in Lowe et al. (2009).

The metabolism of CPF was described as in the SupersomeTM-based PBPK model of Zhao et al. (2022) using Michaelis-Menten kinetics (Supplementary Material 2). Since the metabolism of CPF by A-esterase (PON1) appears to be negligible, we chose to mainly implement the metabolism mediated by CYP450 in the liver for the biotransformation of CPF to CPF-oxon (pathway 1 in Fig. 1) or CPF to TCPy and DETP (pathway 2 in Fig. 1). The main enzyme isoforms involved in these biotransformations are CYP2B6 and 3A4, and the respective Km and Vmax values determined by Zhao et al. (2022) were used. Only two of the four CYP 450 (cytochrome P450) involved in CPF biotransformation were included in our model, namely CYP2B6 and 3A4. In fact, CYP1A2 and 2C19 play a minor role in CPF metabolism (Zhao et al., 2022). PON1 activity was considered in the model only for the biotransformation of CPF-oxon to TCPy and DEP (pathways 3 and 4 in Fig. 1).

In our approach, the placental barrier function is assumed to be non-existent (see section 4), therefore the placental diffusion constant was set to the high value of 1 L/h/dm². Placental diffusion is one order of magnitude higher than placental blood flow. Urinary elimination rates of the CPF metabolites were collected from Zhao et al. (2019) for the

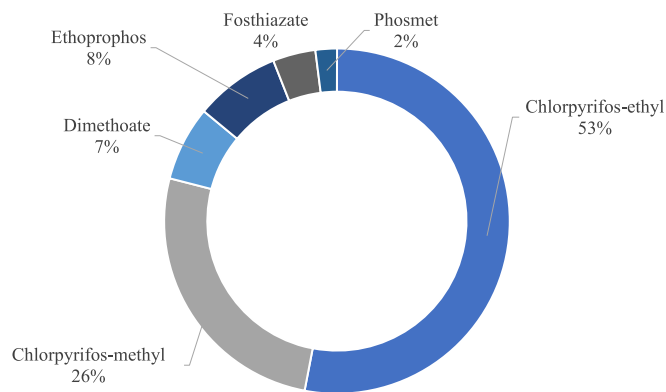


Fig. 4. French organophosphate pesticides sales in 2011 (BNV-D, 2023).

TCPy and from Bouchard et al. (2005) for the DAPs (see equations in Supplementary Material 2).

2.5. Estimation of dietary intake and internal concentration predictions

The steady-state equation was applied to each pregnant woman of the Elfe cohort to estimate their median dietary intake of chlorpyrifos. According to the French National Bank of Distributor Sales (BNVD), chlorpyrifos-ethyl was the major organophosphate sold in France at the time of the sampling in the Elfe cohort (Fig. 4) (BNV-D, 2023). Therefore, it was assumed in a conservative approach that only exposure to chlorpyrifos-ethyl was responsible for the urinary concentrations of DAPs in this work.

The urinary metabolite concentrations were assumed to follow a log-normal distribution with a 2-fold standard deviation. The likelihood of the model given data points below the LOD was calculated by combining both the cumulative log-uniform and log-normal density functions so that the likelihood was almost maximal below 2 geometric standard deviations under the LOD and decreased to near null values over 2 geometric standard deviations over the LOD as in Quindroit et al. (2021). Urinary levels below the LOD are therefore taken into account as such, while the other metabolite level (DEP or DETP) in the sample is also taken into account in the estimation of the exposure dose. In case both DEP and DETP were undetected, the estimated exposure therefore tends towards 0. The likelihood of the model given data points between the LOD and the LOQ (DETP levels, observed in 165 samples) was computed in a similar manner, with the likelihood being maximal between the LOD and the LOQ. To estimate the posterior probability distributions, Markov Chain Monte Carlo simulations were performed by running three independent Markov Chains of 50,000 iterations, recording the last 10,000 iterations. A stable *posterior* distribution was assumed to be reached when the convergence criterion R was below 1.2 (Gelman and Rubin, 1996).

Based on the median value of dietary intake estimates of chlorpyrifos, maternal and fetal internal concentrations were predicted using the p-PBPK model at the end of the pregnancy (at 8.5th months). As CPF affects neurodevelopment in children, predicted fetal brain levels are presented in this work. TCPPy urinary concentrations were also predicted for each woman of the Elfe population assuming constant exposure to CPF. Their urinary flow was set to 0.07 L/h (Van Haarst et al., 2004) and voiding of the bladder was not simulated. Another exposure scenario was modeled, in which the pregnant women from the Elfe cohort were exposed to CPF for 15 min before delivery 3 times a day (modeling of 3 meals between 8 a.m. and 7 p.m.). In this case, 8 urine voids were simulated between 8 a.m. and 10 p.m. (Van Haarst et al., 2004).

2.6. Risk assessment

Estimated dietary intakes of chlorpyrifos were compared to

established TRVs in a risk assessment context. Several agencies and organizations have developed TRVs for CPF. These TRVs are designed for different exposure durations (acute, subchronic and chronic) and guarantee the absence of effects of chlorpyrifos in the general population. The most relevant TRV for the general population to chlorpyrifos exposure was selected (Supplementary Material 3) based on Ineris methodology (INERIS, 2016). An additional, more protective, draft TRV was developed in the present work to protect the most vulnerable sub-populations, including pregnant women and perinatal care. This value was based on literature data specific to neurodevelopment using the Anses methodology (ANSES, 2017). Both TRVs were then compared to the estimated CPF daily intake of the pregnant women from the Elfe cohort.

2.7. Software

Implementation of the PBPK model and exposure estimation was performed using GNU MCSim v6.2.0 (Bois, 2009). R v4.0.2 (R Core Team, 2022) and RStudio 12.0 were used for the data analysis and the figures. The model code is available here: 10.5281/zenodo.11028162. PlotDigitizer (Huwaldt, 2014) was used to collect the data used for model evaluation.

3. Results

3.1. Study population

The population studied in this work is pregnant women from the Elfe cohort. Their main characteristics are described in Table 3. The pregnant women's mean age is 30.0 years old (± 4.8 years old). Their mean weight before pregnancy is 64.7 kg (± 14.1 kg) and after pregnancy is 78.0 kg (± 14.0 kg). The mean duration of pregnancies is 39.3 weeks (± 1.3 weeks). The mean placental weight at birth is 583.7 g (± 133.1 g) and the mean weight of the child at birth is 3360.8 g (± 452.8 g).

3.2. Estimation of dietary intake

The estimation of the CPF dietary intake for each pregnant woman is based on DAPs urinary measurements from the Elfe cohort. Using a steady-state equation for urinary excreted fractions (Equation (1)), an oral CPF exposure level was estimated from the urinary concentrations of DAPs for each of the pregnant women from the Elfe cohort. Urinary excretion predictions were in good agreement with the observations, as shown in Supplementary Material 4. Among the women with at least one quantified level of DEP, 100% of the predictions are within the 10-fold interval, 95% of the predictions are within the 5-fold interval and 58% of them are within the 2-fold interval. Discrepancies reflect the variability of the ratio between measured DEP and DETP levels. Concerning the women with at least one quantified level of DETP, 100% of predictions are within the 5-fold interval and 98% of predictions are within the 2-fold interval. For a large number of women ($n = 696$), both DAP levels in urine were below the LOD or LOQ. For these women the estimates are not reliable since the Markov Chain Monte Carlo (MCMC) chains could not converge since the only information available was that the exposure was beneath a certain level. For the women with urinary

Table 3

Main characteristics of the French pregnant women from the Elfe cohort selected in this study ($n = 899$).

Characteristics	Mean \pm SD
Age (years)	30 \pm 4.8
Maternal weight before pregnancy (kg)	64.7 \pm 14.1
Maternal weight at the end of pregnancy (kg)	78 \pm 14
Gestational age (weeks)	39.3 \pm 1.3
Placental weight (g) ($n = 171$)	583.7 \pm 133.1
Baby weight at birth (g)	3360.8 \pm 452.8

Table 4

Descriptive statistics of estimated chlorpyrifos dietary intake ($\mu\text{g}/\text{kg}$ bw/d) for all the Elfe population considered in this study ($n = 899$) and for the Elfe pregnant women with at least one measured urine concentration of either DEP or DETP ($n = 203$).

	Estimated chlorpyrifos dietary intake for all Elfe pregnant women studied ($\mu\text{g}/\text{kg}$ bw/d) ($n = 899$)	Estimated chlorpyrifos dietary intake for the Elfe pregnant women with at least one DAP measured urine concentration ($\mu\text{g}/\text{kg}$ bw/d) ($n = 203$)
P50	–	0.62
P75	–	1.08
P90	0.66	1.66
P95	1.17	2.30
P99	2.32	5.13
Max	22.1	22.1

P50: 50th percentile, P75: 75th percentile, P90: 90th percentile, P95: 85th percentile, P99: 99th percentile, Max: maximum.

DEP below the LOD and urinary DETP below the LOQ, the estimated exposure was assumed to be less than $0.7 \mu\text{g}/\text{kg}$ BW/day (95th percentile of the upper bound of the 95% credibility interval). For the women with both urinary DEP and DETP levels below the LOD, the estimated exposure was assumed to be below $0.2 \mu\text{g}/\text{kg}$ BW/day.

Table 4 presents the descriptive statistics of the estimated CPF exposure for all the pregnant women ($n = 899$) and the pregnant women for which either urinary DEP or DETP were measured $>$ LOD/LOQ ($n = 203$) from the Elfe cohort using the steady-state equation. The maximum estimated CPF dietary intake is $22.1 \mu\text{g}/\text{kg}$ bw/d, the 95th percentile is $1.17 \mu\text{g}/\text{kg}$ bw/d.

3.3. Predicted maternal and fetal CPF internal concentrations

The exposure estimates were used to predict internal concentrations of pregnant women and fetuses with the developed p-PBPK model at 8.5 months of the pregnancy (Table 5). The predicted median CPF blood concentrations (pregnant women and fetuses: $0.5 \times 10^{-6} \mu\text{g}/\text{L}$) are approximately 10-times lower than the predicted brain concentrations for both pregnant women ($0.44 \times 10^{-5} \mu\text{g}/\text{L}$) and fetuses ($0.42 \times 10^{-5} \mu\text{g}/\text{L}$), as a consequence of the brain: blood partition coefficient value.

3.4. Predicted TCPy concentrations

The CPF exposure estimates were used to predict TCPy urinary concentrations for the Elfe pregnant population using the p-PBPK developed in this study in a forward dosimetry approach. The distribution of the urinary concentration of TCPy among the Elfe cohort is presented in Fig. 5. The predicted minimum and maximum TCPy urinary concentrations are $2 \times 10^{-3} \mu\text{g}/\text{L}$ and $228 \mu\text{g}/\text{L}$, respectively. The median concentration is $6 \times 10^{-3} \mu\text{g}/\text{L}$, the geometric mean concentration is $0.04 \mu\text{g}/\text{L}$ and the arithmetic mean concentration is $2.13 \mu\text{g}/\text{L}$. Since the detection percentage of DAPs in urine is low, the majority of the population had a low concentration implemented of concentrations below the LOD or LOQ and therefore the median concentration of predicted urinary TCPy is lower than the mean predicted concentration. Table S3 summarizes TCPy concentrations measured in different cohorts of pregnant women. The last line of the table indicates the predicted

Table 5

Median CPF blood and brain concentrations ($10^{-6} \mu\text{g}/\text{L}$) in pregnant women and fetuses at the end of the pregnancy (8.5th month).

	Median CPF blood concentration ($10^{-6} \mu\text{g}/\text{L}$)	Median CPF brain concentration ($10^{-6} \mu\text{g}/\text{L}$)
Pregnant women	0.5	4.4
Fetuses	0.5	4.2

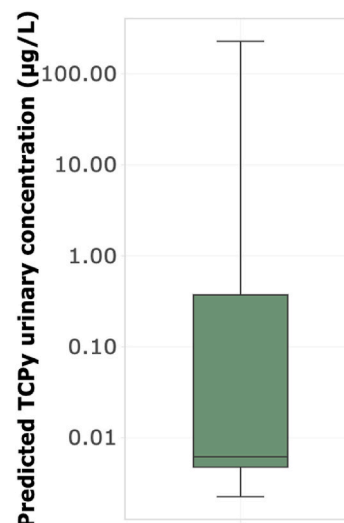


Fig. 5. Box plot representing the distribution of predicted TCPy urinary concentration ($\mu\text{g}/\text{L}$) from the Elfe cohort ($n = 899$).

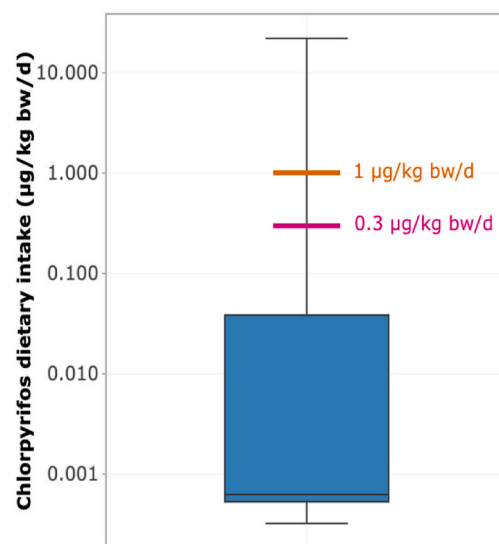


Fig. 6. CPF dietary intake ($\mu\text{g}/\text{kg}$ bw/d) for pregnant women compared to the current TRV of $1 \mu\text{g}/\text{kg}$ bw/d (in orange) and the draft TRV of $0.3 \mu\text{g}/\text{kg}$ bw/d (in pink). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

urinary TCPy concentration for the pregnant women of the Elfe cohort using a conservative assumption.

3.5. Risk assessment

According to the TRV selection methodology presented in the Supplementary Material 3, the recommended TRV for chronic oral exposure to CPF is $1 \mu\text{g}/\text{kg}$ BW/d (ATSDR, 1997). According to our exposure estimation results, 6% of the Elfe pregnant women are exposed above this TRV, which represents a health concern for these individuals (Fig. 6). This value was established taking into account the neurotoxic effect of CPF objectified by measuring the inhibition of the cholinesterase activity in plasma and erythrocytes in rats and concerns for the general population. However, this TRV does not consider the potential neurodevelopmental effects of CPF. The impact of chlorpyrifos on the central nervous system is well characterized, and its effects on children have also been documented. However, the neurotoxic effects of

chlorpyrifos follow several mechanisms of action, and recent studies on neurodevelopment seem to support effects detected at doses lower than those typically used (Gomez-Gimenez et al., 2017, 2018; Silva et al., 2017). Therefore, a neurodevelopmental specific TRV for sub-chronic oral exposure of 0.3 µg/kg bw/d was drafted here from recent literature data, in which rats were exposed to CPF at different doses, from gestational day 7 to postnatal day 21 (Gomez-Gimenez et al., 2017). Learning deficits and spatial learning latency were observed in male rats when exposed to 0.1 mg/kg/d of CPF. Applying an uncertainty factor of 300 (10 for interspecies variation, 10 for differences in sensitivity and 3 for the use of a low observed adverse effect level (LOAEL)), the draft TRV considering neurodevelopmental toxicity is established to 0.3 µg/kg bw/d. If the estimated exposure levels in Elfe pregnant women are considered, 20% of the pregnant women are above the draft TRV of 0.3 µg/kg bw/d for CPF (Fig. 6).

4. Discussion

The main objective of this work was to estimate the exposure of French pregnant women to CPF using urinary DAPs in 2011, before the CPF ban. Since we were assumed to be in a steady-state and a constant exposure condition, a steady-state equation was used to estimate external exposure to CPF based on urinary metabolite levels. The results obtained under these conditions are equivalent to those obtained using the PBPK model for such a low persistent compound as CPF (Tarazona et al., 2022b).

The steady state hypothesis can be considered as CPF is a relatively low persistent compound to which the population was exposed daily through diet before its ban. Most organophosphate pesticides produce DAPs (75% of them) (Jain, 2016) and some of them are metabolized to DETP and DEP (e.g., diazinon or parathion) (Sudakin and Stone, 2011). Therefore, using DETP and DEP as HBM data to characterize CPF exposure (conservative assumption) leads to an overestimation of the exposure, even if CPF is the main organophosphate pesticide sold in France in 2011. Exposure estimates are potentially overestimated due to the exposure to both CPF and CPF-methyl (Fig. 4), which also produce TCPy, DETP and DEP urinary metabolites. In this work, the estimation of the exposure to CPF based on the DAPs measurement in urine remains a conservative approach and could be overestimated by around 30% based on CPF-methyl sales in France (Fig. 4).

However, our estimates under this conservative assumption are close to the results of other studies (Algharably et al., 2023; Taheri et al., 2022). Taheri et al. (2022) estimated CPF exposure for a pregnant Iranian population based on an equation similar to the one used here, taking into account urinary TCPy concentration, urine volume, urinary excretion of TCPy, molecular weights, and women's body weight. The estimated daily dietary intakes were in the same range as those estimated in our study (mean: 0.17 ± 0.13 µg/kg bw/d, P50: 0.15 µg/kg bw/d and P95: 0.39 µg/kg bw/d). In a recent study, CPF exposure of pregnant women from different cohorts were estimated using a p-PBPK model based on existing biomonitoring data (Algharably et al., 2023). In two of these cohorts, exposure to CPF was estimated at 0.11 µg/kg bw/day (Rauh et al., 2011) and 0.2 µg/kg bw/day (Rauh et al., 2006), which are in the same range as those estimated in our study (Algharably et al., 2023).

In the French pregnant women, DAPs were not detected in most samples. Several cohorts with similar urinary HBM data measured DAPs concentrations above the LOD (Abdeen et al., 2016; Wang et al., 2017; Ye et al., 2008). This may suggest that the French pregnant women of the Elfe cohort could be exposed to the parent substances of DAPs, but it was not detected in the urine samples. The detection rate for DEP (4%) or DETP (21%) in urine samples in the Elfe cohort is lower than the detection rate observed in other cohorts (Ye et al. (2008): >96%, Abdeen et al. (2016): 98.6%, Wang et al. (2017): >97.6%). The inter-individual variability of the estimated exposure among the Elfe cohort is important and could be explained by the data below the LOD or

the LOQ, although the inter-individual variability remains important even among pregnant women with at least one DAPs measured above LOD/LOQ (Table 4). Data below LOD or LOQ were taken into account in our calculations without being replaced by a set value (e.g. LOD divided by 2), by considering the information this data carries, i.e. an upper bound for these values. The data cannot therefore not inform about low percentiles of exposure estimates, but the highest estimates, obtained from the 22% of samples where a DAP was detected, can be considered in a risk assessment perspective. CPF exposure routes are multiple (ANSES, 2011; Désert et al., 2018; Deziel et al., 2017; Deziel et al., 2013). Pregnant women with the highest exposure estimates could have been exposed to CPF through multiple exposure routes, and therefore have a higher exposure to CPF due to their occupation, proximity to application sites, or even dietary habits.

The p-PBPK developed in this study was used to predict TCPy urinary concentrations for the Elfe pregnant women using a forward dosimetry approach based on the estimated CPF dietary exposure. Our method of taking into account values below the LOD allows a refined estimation of the dietary intake of CPF and consequently of urinary TCPy levels in the pregnant population of the Elfe cohort. The measured DAP levels in the Elfe cohort are not consistent with the levels of TCPy measured in other cohorts, assuming that the CPF metabolic rates in the two concurrent pathways described in this paper are realistic, and assuming that the metabolites are rapidly excreted. The 95th percentile and the mean of the predicted urinary TCPy concentrations are in the same range as the observed 95th percentile and mean of the observed urinary TCPy concentrations observed in other countries: Netherlands (Ye et al., 2008), Denmark (Dalsager et al., 2019) and Norway (Ye et al., 2009) (Supplementary Material 6). The highest predicted urinary TCPy concentrations in the Elfe cohort are in the same range as other highest levels in European cohorts of pregnant women (Dalsager et al., 2019; Ye et al., 2008). Despite the fact that chlorpyrifos has been banned from the European Union (EU) in 2020 and was previously restricted in 2016, its specific metabolite TCPy is still detected in urine (EFSA, 2023; Makris et al., 2022; Sulc et al., 2022). Besides, several countries outside the EU still use CPF, and therefore CPF exposure in the EU may still be a concern due to imports.

Human biomonitoring data associated with PBPK modeling is a robust approach to ensure a better hazard characterization to support risk assessment (EFSA Scientific Committee, 2019). However, shifting from maternal urinary biomarkers to fetal internal exposure is challenging and depends on the available data. In our work, a chlorpyrifos p-PBPK model was developed to characterize maternal and fetal exposure to CPF among pregnant women of the French national Elfe cohort based on urinary biomarker data. To this end, a previous adult PBPK model (Zhao et al., 2022) for CPF and its metabolites (i.e. CPF-oxon, TCPy, DEP and DETP) was adapted to consider pregnancy by incorporating maternal organ growth and development of the foeto-placental unit. Internal predictions in adults were consistent with literature data (Supplementary Material 5). Since one of the aims of our work was to predict the fetal concentration of CPF, the fetus was modeled with a compartmental structure including the brain as it is a target organ, as well as other key compartments, unlike other available p-PBPK models, that describe the fetus as only one compartment (Smith et al., 2014) or only describing the brain and the rest of the fetal body (Algharably et al., 2023). Due to the lack of quantitative information in pregnant women for CPF, we did not include an effect of pregnancy on partition coefficients or metabolic clearances. According to common practice in p-PBPK modeling in absence of data, fetal partition coefficients were set to those of the mother (Thépaut et al., 2023). A p-PBPK model developed for CPF has been published previously (Algharably et al., 2023), with partition coefficients based on the algorithm of Schmitt (Schmitt, 2008). These partition coefficients are higher than those used in this study, and simulated brain CPF concentrations were approximately 100 times higher than those in blood. In our study, the predicted brain CPF concentrations are approximately 10 times higher than in blood based on

partition coefficients calculated by the DeJongh method based on the logP value (DeJongh, 1997; Zhao et al., 2019). Our model was validated for the non-pregnant population based on human studies (Supplementary Material 5), as performed in Zhao et al. (2019). Additionally, more data on DAPs excretion following a chlorpyrifos exposure are needed to better understand the variability in the ratio between DEP and DETP urinary levels. In the current PBPK model, the DAP elimination rates are set by default to the value determined by Bouchard et al. (2005), i.e. a toxicokinetic model which considers the excretion of DAPs directly from the CPF blood concentration. This default value is sufficiently high to allow a rapid excretion and achievement of steady-state urinary concentrations. Knowledge on the kinetics of the excretion of DEP and of DETP would allow an estimation of the kinetics of DEP and DETP excretion under more realistic, non-constant exposure scenarios.

The structure of the PBPK model is based on well-established models for CPF and for pregnancy. However, due to lack of data enabling characterization of chlorpyrifos placental transfer throughout pregnancy the application of this model is restricted to a worst-case scenario representing the absence of placental barrier. The only data on the placental transfer in humans are available for the end of the pregnancy (Whyatt et al., 2009), but not during the entirety of pregnancy. This data reveals that at the end of pregnancy, fetal blood CPF levels are equivalent to the maternal levels. The equations used for the placental transfer in the p-PBPK model imply increasing exchanges as the exchange surface increases. The available data for CPF does not unfortunately provide information on whether the permeability is maximal from the onset of pregnancy or whether it increases gradually throughout pregnancy. As the placenta is an organ with a key role throughout the pregnancy (e.g., gas exchange, elimination of waste, nutrients transfer, hormone synthesis and also acts as a barrier to protect the fetus), missing data in the first months of pregnancy for this compartment leads to higher uncertainties in our models for this period. Thus, without additional data on CPF placental transfer, we recommend to use the current p-PBPK model only at the end of the pregnancy to predict internal or external exposures as a worst case scenario. Indeed, passive diffusion of chemicals through the placental barrier is affected by their physicochemical properties (i.e., molecular weight, lipophilic profile) (Allegaert and Van Den Anker, 2017; Codaccioni and Brochot, 2020). In addition, the transfer rate of a substance across the placenta depends on its permeability, thickness of the membrane and surface of exchange. As CPF has a low molecular weight, few hydrogen binding sites and is lipophilic, it appears that this chemical easily crosses the placental barrier. The physicochemical properties of CPF also make it a good candidate for a rapid transfer rate across the placenta and a rapid distribution in fetal organs. Therefore, concentrations in fetal blood would rapidly rise to reach maternal blood concentrations until equilibrium is reached (Allegaert and Van Den Anker, 2017). In this case, the placenta does not act as a barrier to protect the fetus from CPF exposure as it crosses easily and rapidly the placenta, explaining why maternal and fetal CPF internal concentrations are within the same range at the end of pregnancy. Human data suggest that chlorpyrifos crosses the placenta easily and maternal and fetal blood concentrations reach an equilibrium at the end of pregnancy (Whyatt et al., 2009). However, an experiment in pregnant rats (gestational days 11–13, approximately half the duration of pregnancy) suggests that the placenta acts as a barrier for CPF since fetuses CPF concentrations are lower (from an approximate factor 20) than those observed in the pregnant rat plasma and the fetal plasma (Abdel-Rahman et al., 2002). These data suggest a variation of the CPF placental transfer during the pregnancy. Therefore, further investigations on CPF placental transfer need to be conducted for a better characterization of CPF transfer through the placenta. If data on CPF placental transfer suggests that the placenta acts as a barrier at the early stages of pregnancy, they could be implemented into the model and allow better characterization of fetal kinetics at the beginning of pregnancy where neurodevelopment key-events occur (Semple et al., 2013). Furthermore, characterizing fetal exposure to CPF is challenging due to

sparse measurements of fetal CPF internal concentrations. The CPF specific metabolite, TCPy, can cross the placenta as it has been previously measured in rat fetuses (Abdel-Rahman et al., 2002). To our knowledge, no fetal data for TCPy in humans is available in the scientific literature. Besides, the toxic metabolite CPF-oxon is not currently included in the fetal part of our model. CPF-oxon is rapidly metabolized into DEP and TCPy (Fig. 1), making it difficult to quantify and to characterize CPF-oxon placental transfers. Additionally, CPF-oxon may cross the placenta and be metabolized by the fetus *in utero*. Knowledge of fetal metabolism remains sparse but could be considered and integrated into the p-PBPK model if additional data are provided.

In addition, although exposure estimates from the literature were within the same range as our estimations, the predicted fetal brain concentration was higher than our predictions (predictions of 0.85 µg/L and 1.75 µg/L for Rauh et al. (2006, 2011), respectively). Current limitations of our model, due to the lack of data regarding the fetal kinetics aspects, can introduce additional uncertainties on the CPF predictions. Further investigations should be carried out to ensure a better understanding of fetal exposure.

Moreover, CPF has been described previously as having a relatively high potential to permeate the human brain (Cresto et al., 2023). It has also been suggested that CPF exposure might impair the functionality of the blood-brain barrier (BBB) (Deepika et al., 2022). The BBB has been shown to be effective from gestation week 8 onwards (Goasdoue et al., 2017). However, some BBB transporters are more or less effective in the developing fetus, and fetal blood vessels are more fragile, which may increase susceptibility to xenobiotics (Saunders et al., 2012) particularly in the first weeks of gestation. Neurodevelopment can be divided into several phases (Semple et al., 2013). During the first few weeks of fetal development, neurogenesis takes place (i.e. the creation of neurons) (Semple et al., 2013). This stage of neurodevelopment, which lasts until the individual is 1 year old, constitutes a window of sensitivity to xenobiotics. Because of its characteristic as a good candidate for higher permeation and the BBB development during pregnancy, CPF could reach the fetal brain more easily than in adults particularly if placental transfer occurs to a high extent. Further research is therefore necessary to determine the changes that occur at the cellular and tissue level in humans and animals at all stages of life, and to investigate how they will affect the body several years after exposure to CPF (Wolejko et al., 2022)

Finally, exposure estimates for pregnant women were compared to existing and draft TRVs, considering neurodevelopmental toxicity, to characterize the potential CPF related risk of these populations. Among pregnant women in the ELFE cohort, 6% (54 pregnant women) are exposed above the current TRV value. This current TRV value is based on inhibition of acetylcholinesterase activity in rat blood and plasma by CPF. While acetylcholinesterase inhibition is indeed a known biomarker of the neurotoxic effects of organophosphorus compounds, the mechanisms have been shown to involve other pathways of effects that must also be considered to protect against the full range of neurotoxic effects (Burke et al., 2017). Individuals with exposure to CPF above the current TRV are at risk of developing pathologies linked to the effects of CPF such as neurotoxic effects. Because it has been demonstrated that fetal exposure to xenobiotics can lead to adverse effects at lower concentrations (Falck et al., 2015), this risk could be higher for fetuses.

In order to take into account increased prenatal sensitivity, a draft sub-chronic TRV was proposed in this work based on neurodevelopmental toxicity of CPF. The draft TRV was derived from recent data obtained from an *in vivo* study (Gomez-Gimenez et al., 2017). Rats were exposed to chlorpyrifos from gestational day 7 to postnatal day 21, at different doses (0.1, 0.3 and 1 mg/kg/day). At 2–3 months, the pups were subjected to spatial learning and memory tests. From the lowest dose, an impairment in escape latency and learning are observed in the Morris water maze test. At the highest dose, impairments in learning and memory are observed for both mazes used in the study. The draft TRV is established from the lowest exposure dose in this study, with the application of an uncertainty factor of 300, resulting in a draft TRV of

0.3 µg/kg bw/d. Other studies conducted on neurodevelopment in rats confirm the occurrence of alterations in motor activity or anxiogenic-type effects for similar levels of exposure (Gomez-Gimenez et al., 2018; Silva et al., 2017).

Taking into account the calculated exposures, 20% (180 pregnant women) of pregnant women are exposed to CPF levels that exceed the draft TRV. Thus, the estimated exposure of pregnant women raises concerns that neurodevelopmental effects of CPF could be observed in this population. Fetuses described here are a part of the Elfe cohort and therefore are followed throughout childhood. It would be interesting to assess whether these individuals are subject to neurodevelopmental impairments over the years, in a context of DOHaD concept, as it was assessed in Sagiv et al. (2021).

5. Conclusion

Human biomonitoring data from the Elfe cohort were used to estimate the dietary intake of CPF in pregnant women, assuming continuous constant exposure and steady-state kinetics, by using the fraction of the parent compound excreted as metabolites in urine. A p-PBPK model was adapted from an existing adult model specific for CPF and from existing pregnancy PBPK models. This model integrates the excretion of CPF urinary metabolites TCPy, DEP and DETP. The internal concentrations (blood, brain for CPF -mother and fetus- and urine TCPy mother) were then predicted for pregnant women and fetuses using the p-PBPK model. Finally, the estimated dietary intake of CPF by French pregnant women in the Elfe cohort was compared with a current toxicological reference value (TRV) and a draft TRV. The results highlighted that the exposure of some pregnant women to CPF (6%) was above the current TRV established for adults (or the general population), as much as 20% of the pregnant women were overexposed compared to the draft TRV. This observation raises health risk concerns and further investigation could be undertaken to characterize the potential effects of CPF exposure during fetal development at the realistic exposure levels determined in the present study, especially as CPF exposure may still occur despite its ban in Europe.

CRedit authorship contribution statement

Elisa Thépaut: Writing – original draft, Software, Methodology, Investigation. **Cleo Tebby:** Writing – review & editing, Validation, Software, Methodology, Investigation, Conceptualization. **Michèle Bisson:** Writing – review & editing, Validation, Methodology, Investigation. **Céline Brochot:** Writing – review & editing, Methodology, Investigation. **Aude Ratier:** Writing – review & editing, Software, Methodology. **Cécile Zaros:** Writing – review & editing, Methodology. **Stéphane Personne:** Writing – review & editing, Investigation. **Karen Chardon:** Writing – review & editing, Investigation. **Florence Zeman:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2024.114480>.

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