



Full length article

Mixture risk assessment of pesticides with acute neurotoxic effects: a novel approach based on urinary exposure biomarkers

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ABSTRACT

Pesticides showing neurotoxic effects, including organophosphates, pyrethroids, and N-methyl carbamates, are widely used in agriculture. Human biomonitoring (HBM) integrates exposure from multiple sources and routes by measuring pesticide levels in human biological samples. This study applied a novel MRA methodology using urinary biomarkers, focusing on two cumulative assessment groups (CAGs) of pesticides with acute neurotoxic effects, as previously established by the European Food Safety Authority (EFSA), namely, CAG-NAN on brain and/or erythrocyte acetylcholinesterase inhibition; and CAG-NAM on functional alterations of the motor, sensory and autonomic division. Available urinary pesticide metabolites were assigned to potential parent compounds within these CAGs and were converted to external exposure levels, expressed as estimated daily intakes (EDI), using reverse dosimetry. Toxicological reference points, i.e., no observed adverse effect levels (NOAEL) were obtained from EFSA report. For each parent compound, the ratio of the NOAEL to the EDI was calculated, and these ratios were used to

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<https://doi.org/10.1016/j.envint.2026.110531>

Received 16 June 2026; Received in revised form 16 September 2026; Accepted 16 September 2026

Available online 20 September 2026

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determine the total margin of exposure (MOET). This approach was applied as a proof-of-concept to children and adults from 10 HBM studies across seven European countries. For CAG-NAN, median MOET ranged between 27 and 2485 across datasets with percentage of individuals below a threshold of 100 from 2.8 to 97%. For CAG-NAM median MOET values ranged from 34 to 220 000 and in one scenario the percentage of individuals below the threshold was between 24% for children and up to 93% for adults. Azinphos-ethyl, triazophos, omethoate, and chlorpyrifos were identified as risk drivers. This study highlights the need for improved MRA methodologies based on HBM data, including the development of pesticide-specific exposure biomarkers, and the establishment of human biomonitoring-guidance values (HBM-GVs). Incorporating HBM data strengthens human health MRA by reducing uncertainty surrounding non-dietary exposure and generating valuable evidence to inform future pesticide monitoring and regulatory strategies.

Nomenclature		FLEHS 1, 2, 3 and 4 Flemish environmental and health studies 1, 2, 3 and 4	
3xG	Health – Municipalities – Births	FR	France
AchE	acetylcholinesterase	HBM	Human biomonitoring
AMOE	Acceptable margin of exposure	HBM-EL	Human biomonitoring exposure level
BE	Belgium	HBM-GV	Human biomonitoring-guidance value
CAG	Cumulative assessment group	LOD	Limit of detection
CELSPEC: SPECIMEn	Central European Longitudinal Study of Parents and Children: Survey on PEstiCide Mixtures in Europe	LOQ	Limit of quantification
CZ	Czech Republic	MCRA	Monte Carlo Risk Assessment
DAP	Dialkylphosphates	MOE	Margin of exposure
DCCA	3-(2,2-dichlorovinyl)-2,2dimethylcyclopropanecarboxylic acid	MOET	Total margin of exposure
DE	Germany	MRA	Mixture risk assessment
DEP	Diethylphosphate	NAM	Functional alterations of motor division
DMP	Dimethylphosphate	NAN	Acetylcholinesterase inhibition
EDI	Estimated daily intake	NL	The Netherlands
EFSA	European food safety authority	NOAEL	No observed adverse effect level
ES	Spain	PARC	Partnership for the assessment of risks from chemicals
ESB	Environmental specimen bank	PBK	Physiologically based kinetic
ESTEBAN	Health study on the environment, biomonitoring, physical activity and nutrition	PPP	Plant protection products
EU	European Union	SI	Slovenia
EXHES-ES	Exposure and health examination survey Spain	SLO-CRP	Exposure of Children and Adolescents to Selected Chemicals Through Their Habitat Environment (Slovenia)
		SPECIMEn-NL	Dutch arm of the HBM4EU pesticide study SPECIMEn (The Netherlands)
		WHO	World Health Organisation

1. Introduction

Pesticides refer to compounds aimed at controlling unwanted organisms (insects, rodents, fungi, etc.) that pose risks to crops, animals and humans. They are used in the formulation of the respective plant protection products (PPP) to protect crops during primary production or post-harvest. PPPs are composed of several families of active substances, for example, N-methyl carbamates, organophosphates, and pyrethroids, which are commonly used as insecticides. Since the 1970s, organophosphates and pyrethroids have been widely used in Europe as active insecticidal substances in PPPs for agricultural uses. Organophosphates, known for their efficacy against a broad spectrum of insects, were extensively applied in agriculture to protect crops such as cereals, fruits, and vegetables. Nowadays, most organophosphate insecticides are no longer approved for use in the European Union (EU), with chlorpyrifos and chlorpyrifos-methyl withdrawn in 2020 (Regulation (EU) 2020/18). Pyrethroids, synthetic analogues of the natural pyrethrins, emerged as a preferred alternative to other insecticides due to their relatively low mammalian toxicity, and favourable environmental fate, including limited mobility and rapid degradation. In addition to their agricultural applications, both organophosphates and pyrethroids have also been used in biocidal products for the control of insects. They have been used both indoors and outdoors in residential areas to control mosquitoes, ticks and lice, replacing the previously used organochlorine pesticides. N-methyl carbamates were introduced as an alternative to organophosphates in the 1950s. These compounds are derived from carbamic

acid and share the same molecular target as organophosphates, i.e., acetylcholinesterase (AChE), which plays a critical role in nervous system function.

Human health risk assessment from exposure to pesticides is generally conducted using estimates of external dietary exposure, as recommended by regulatory authorities such as the European Food Safety Authority (EFSA) and the World Health Organisation (WHO) (Kruisselbrink et al., 2025; FAO/WHO, 2024). This approach integrates food consumption data with pesticide residue concentrations measured in different food commodities (occurrence data) to estimate dietary intake and assess the associated potential health risks. Occurrence data on parent compounds and/or their metabolites (defined according to the regulatory residue definitions for monitoring) are primarily derived from national pesticide residues monitoring and control programmes, alongside multiannual EU-level control programmes. With respect to the assessment of pesticide mixtures, based on acute effects of pesticides on the nervous system, EFSA defined cumulative assessment groups (CAGs) for pesticides based on five effects on the nervous system. Two of them were selected for this case study: 1) 47 substances associated with brain and/or erythrocyte acetylcholinesterase inhibition (CAG-NAN); and 2) 100 substances associated with functional alterations of the motor, sensory and autonomic division (CAG-NAM) (EFSA, 2019b). After populating these CAGs with pesticides meeting the established inclusion criteria, EFSA developed a methodology for conducting retrospective mixture risk assessment (MRA) of pesticide residues in food (referred to in EU legislation as cumulative risk assessment, CRA) (EFSA, 2013). Retrospective MRA aims to estimate the possible health risks for

populations during a recent past period. Based on those CAGs, EFSA performed retrospective acute MRA of dietary exposure for 10 European populations: Danish, Dutch and British toddlers (≥ 12 months– < 36 months); Bulgarian, French and Dutch children (≥ 36 months– < 10 years); and Belgian, Czech, German and Italian adults (van Klaveren et al., 2019; EFSA, 2019a).

As an alternative approach to external dietary exposure data, human biomonitoring (HBM) studies quantify chemicals, including pesticide parent compounds and/or their metabolites, in human biological samples such as blood, urine, or hair. These measurements integrate all sources and routes of exposure, including dietary intake, inhalation, and dermal absorption. Upon absorption, organophosphates undergo biotransformation leading to the formation of dialkylphosphates (DAP), non-specific metabolites which are excreted in urine (Buratti et al., 2003). Most organophosphates are metabolised into either diethyl phosphates (DEP) or dimethyl phosphates (DMP). Similarly, pyrethroids are metabolised to several common metabolites such as 3-phenoxybenzoic acid (3-PBA) or 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA), as well as compound-specific metabolites, for example *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DBCA), which is specific to deltamethrin (Ueyama et al., 2010; Lu et al., 2019). In the case of N-methylcarbamates, carbofuranphenol is a metabolite produced during the biotransformation of carbofuran, carbosulfan and benfuracarb (Petropoulou et al., 2006). As a consequence, only for a few limited number of pesticides, specific exposure biomarkers are available, such as 3,5,6-trichloro-2-pyridinol (TCPy) for chlorpyrifos and chlorpyrifos-methyl (and for the herbicide triclopyr), 4-Fluoro-3-phenoxybenzoic acid (4-F-3-PBA) for cyfluthrin and beta-cyfluthrin, and *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DBCA) for deltamethrin. In contrast, common metabolites derived from different parent compounds are widely used as exposure biomarkers in urine, such as DAP metabolites (DEP, DETP, DEDTP, DMP, DMTP, and DMDTP) for organophosphates, as well as 3-PBA, *cis*- and *trans*-DCCA for pyrethroids. Consequently, attributing measured biomarker levels to specific parent compound remains a significant challenge in pesticide risk assessment. To interpret internal biomarker levels, corresponding internal toxicological values are necessary. To date, human biomonitoring-guidance values (HBM-GVs) have been established for chlorpyrifos, deltamethrin, and cyfluthrin (Arnold et al., 2015; Apel et al., 2023).

Within the EU-funded Partnership for the Assessment of Risks from Chemicals (PARC) (<http://www.eu-parc.eu/>), the Real-life Mixtures project aims to develop methods and fill data gaps for MRA using HBM data (Crépet et al., submitted). The present study sought to develop an HBM-based MRA approach for pesticides with acute effects on the nervous system, and to compare the resulting risk estimates with those obtained using established MRA approaches based on external dietary exposure data, such as those applied by EFSA. This approach was applied as a proof-of-concept to population groups of children and adults using HBM datasets from seven European countries: Belgium (BE), Czech Republic (CZ), Germany (DE), France (FR), Slovenia (SI), Spain (ES), and the Netherlands (NL).

2. Methods

Internal exposure data were collected from European HBM studies including measurements pesticide biomarkers (i.e., metabolites) in urine (Section 2.1). For both CAGs, starting from the available biomarkers measured in HBM studies and informed by expert judgment and scientific literature, parent compounds from which the metabolites may originate, were identified (Section 2.2). External exposure estimates were derived at the individual level using a reverse dosimetry approach (Section 2.3). For the risk characterisation, and in line with EFSA's methodology, the total margin of exposure (MOET) was calculated for different population groups (Section 2.4). The analyses are performed at the individual level using the Monte Carlo Risk Assessment (MCRA)

toolbox (Section 2.6). Finally, the results are compared to the MRA performed with external exposure data by EFSA (Section 2.7).

2.1. Human biomonitoring data

Ten European HBM studies from seven countries measuring pesticide metabolites in urine were organised and harmonised using the HBM data toolkit (<https://hbm.vito.be/tools/data-harmonization>) (Peeters et al., 2025). The following datasets were included: 3xG (BE), CELSPAC: SPECIMEn (CZ), ESB (DE), ESTEBAN (FR), EXHES-ES (ES), FLEHS 2 (BE), FLEHS 3 (BE), FLEHS 4 (BE), SLO-CRP (SI), and SPECIMEn-NL (NL). In general, the included studies were heterogeneous and covered various study designs, pesticide metabolite biomarkers and target populations (Table 1). ESB (DE) is a repeated cross-sectional study on young adults from the general population, ongoing since the early 1980s, and a subset of data from the most recent years (2015–2020) was provided for the current research. FLEHS (BE) is a repeated cross-sectional study covering different age groups of the general population; and data from FLEHS 2, 3 and 4 were included in this analysis. ESTEBAN (FR), SPECIMEn-NL (NL), CELSPAC: SPECIMEn (CZ) and SLO-CRP (SI) are cross-sectional studies focusing on various groups of the general population. 3xG (BE) and EXHES-ES (ES) are both longitudinal studies. Urine samples in all HBM datasets consisted of spot or first-morning urine samples, except for ESB (DE), where 24-h urine samples were collected.

Urinary concentrations of pesticide exposure biomarkers were standardized for creatinine to account for urinary dilution by dividing individual biomarker concentrations by the corresponding individual creatinine concentration. Participants without available creatinine measurements were excluded from the analysis.

In relation to left-censored data treatment, samples analysed below the limit of detection (LOD) were replaced by zero, while those below the limit of quantification (LOQ) were replaced by half the LOQ, in accordance with EFSA's Tier I approach (EFSA, 2019a). For ESB (DE) and 3xG (BE), LOD values were not available, and for FLEHS 2 (BE), FLEHS 3 (BE), SLO-CRP (SI), and EXHES-ES (ES), LOQ values were not available. In such cases, all non-detects were treated using the same substitution approach described above. In this study, data on two population groups were considered for the analysis: children from 6 to 17 years old and adults from 18 years to 75 years.

2.2. Selection of the parent compounds

In the available HBM datasets, pesticide biomarkers (i.e., metabolites) were measured in urine, whereas the parent compounds themselves were not directly quantified. Most of the available biomarkers were common metabolites to several parent compounds (i.e., 3-PBA, DEP, DMP, DETP, DMTP, etc.). Thus, the measured metabolites had to be associated to specific parent compounds of the CAG of concern which appears to be a significant challenge.

In this study, parent compounds were associated with urinary metabolites into which they can be metabolized, using toxicokinetic information on metabolism and urinary excretion of metabolites derived from parent pesticides included in CAG-NAN and CAG-NAM. This information was gathered from the scientific literature or reviews (Chang et al., 2009; Yusa et al., 2015; Forsberg et al., 2011; Hernández et al., 2019), as well as from institutional reports such as EFSA (EFSA, 2016) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) documents (FAO/WHO, 2002). When a parent compound could be linked to one or more metabolites measured in the HBM datasets, it was selected for inclusion in the analysis. Parent compounds included in the EFSA CAGs for which no corresponding urinary metabolites were measured in any of the 10 HBM datasets were excluded from the MRA. As a result, the number of pesticides included in the analysis varied across datasets.

Table 1

Description of the 10 European HBM datasets used in the mixture risk assessment.

Dataset name	Study design	Population group	Country code	Urine samples	Sampling period	N	Age range (years)	Selected biomarkers	Reference
3xG	Longitudinal	Children	BE	Morning urine	2019–2020	110	6–8	TCPy, <i>cis</i> -DCCA, ClFCA, <i>cis</i> -DBCA, 4-F-3-PBA, <i>trans</i> -DCCA, 3-PBA	Govarts et al. (2020)
CELSAC: SPECIMEn	Cross-sectional	Children	CZ	Spot urine	2019–2020	110	4–15	3-PBA, t/c-DCCA, TcPy,	Sulc et al. (2022)
ESB	Repeated cross-sectional	Adults	DE	24 h urine	2015–2020	180	20–29	DEP, DETP, DEDTP, DMP, DMTP, DMDTP, TcPy, 3-PBA, <i>cis</i> -DBCA, <i>cis</i> -DCCA, <i>trans</i> -DCCA, 4-F-3-PBA	Kolossa-Gehring et al. (2012)
ESTEBAN	Cross-sectional	Children	FR	Morning urine	2014–2016	101	6–10	DMP, DEP, DMTP, DETP, DMDTP, DEDTP, TcPy, <i>cis</i> -DBCA, <i>trans</i> -DCCA, <i>cis</i> -DCCA, 3-PBA, 4-F-3-PBA, Carbofuranphenol	Tagne-Fotso et al. (2023)
EXHES-ES	Longitudinal	Adults [Pregnant women]	ES	Spot urine	2016	61	18–45	DEAMPY, TcPy, 4-F-3-PBA, 3-PBA	Bravo et al. (2020)
FLEHS 2	Cross-sectional	Children	BE	Morning urine	2008–2009	144	13–16	DMP, DEP, DMTP, DETP, DMDTP, DEDTP	Croes et al. (2015)
		Adults				140	23–40		
FLEHS 3	Cross-sectional	Adults	BE	Spot urine	2014	184	50–65	DMP, DEP, DMTP, DETP, DMDTP, DEDTP, <i>trans</i> -DCCA, <i>cis</i> -DCCA, <i>cis</i> -DBCA, TcPy, 3-PBA	Schoeters et al. (2017)
FLEHS 4	Cross-sectional	Children	BE	Spot urine	2017–2018	424	13–16	TcPy, 3-PBA	Schoeters et al. (2022)
									De Troeyer et al. (2022)
SLO-CRP	Cross-sectional	Children	SI	Morning urine	2018	149	7–10	TcPy, 3-PBA, 4-F-3-PBA	Stajniko et al. (2020)
SPECIMEn-NL	Cross-sectional	Children	NL	Spot urine	2020	88	6–11	TcPy, <i>trans</i> -DCCA, 3-PBA, <i>cis</i> -DCCA,	Sulc et al. (2022)
		Adults				105	30–56	ClFCA, <i>cis</i> -DBCA, F-PBA	

Abbreviations: 3-PBA: 3-phenoxybenzoic acid, 4-F-3-PBA: 4-fluoro-3-phenoxybenzoic acid, BE: Belgium, *cis*-DBCA: *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *cis/trans*-DCCA or t/c-DCCA: *trans/cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, CZ: Czech Republic, DE: Germany, DEAMPY: 2-diethylamino-6-methylpyrimidin-4-ol, DEDTP: Diethyldithiophosphate, DEP: Diethylphosphate, DETP: Diethylthiophosphate, DMDTP: dimethyl dithiophosphate, DMTP: Dimethylthiophosphate, DMP: Dimethylphosphate, ES: Spain, FR: France, N: Number of individuals included in the dataset, NL: the Netherlands, SI: Slovenia, TcPy: 3,5,6-trichloro-2-pyridinol.

2.3. Estimation of external exposure

External exposure estimates were derived at the individual level using a reverse dosimetry approach applied on HBM data. Internal urinary biomarker concentrations were converted into external estimated daily intake (EDI) values using steady-state equations commonly used in physiologically based kinetic (PBK) models to predict internal kinetics over time for both metabolites and parent compounds. The steady-state equation, also called a mass balance equation, assumes a linear relationship between the excretion rates of major metabolites and ingestion rates under steady-state conditions (Thépaut et al., 2025). To account for the possibility that a given biomarker may originate from several pesticides, the probability of occurrence of each parent compound and its relative contribution to the urinary biomarker concentration were estimated based on pesticide occurrence patterns and dietary exposure data from the residue monitoring programs conducted at the national level and organised by EFSA.

2.3.1. Estimation of potential presence

Diet was assumed to be the main source of pesticide metabolites measured in urine. The potential presence represents the probability that a given parent compound is present as a pesticide residue in foods, conditional on the detection of its associated metabolite. In other words, it represents the fraction of the measured urinary metabolite concentration that could plausibly originate from a specific parent compound. Potential presence values were estimated using two alternative approaches, depending on data availability per country. First, from dietary exposure data (number of simulated individuals with a positive dietary exposure to a given parent compound in the distribution). Second, when such exposure distributions were not available, potential presence was

estimated from pesticide occurrence patterns in food commodities. In this study, in order to take this probability into account in the reverse dosimetry approach, this potential presence was applied.

2.3.1.1. Potential presence using dietary exposure data. In the EFSA acute retrospective MRA for CAG-NAN and CAG-NAM, dietary exposure data (defined as the number of simulated individuals with a positive dietary exposure to each parent compound) were available for French children, Belgian, German and Czech adults (van Klaveren et al., 2019). In that assessment, EFSA estimated probabilistic cumulative dietary exposure to pesticides (parent compounds) using national food consumption data and pesticide occurrence data from Europe in the period of 2014–2016. Exposure distributions were generated using 100,000 Monte Carlo simulations. In the present study, using EFSA Tier II calculations, potential presence values were calculated for each parent compound–metabolite combination and for each country as:

$$PP_{k,m}(\%) = \frac{n_k}{N_m} \times 100 \quad (a)$$

Where $PP_{k,m}$ is the potential presence of metabolite m originating from parent compound k (%), n_k is the number of simulated individuals with positive exposure to parent compound k , and N_m is the total number of simulated individuals with positive exposure to all other parent compounds assumed to be metabolised into metabolite m .

This approach was used to calculate the potential presence values for the following HBM datasets and their respective population groups: ESTEBAN children (FR), FLEHS 2 and 3 adults (BE), ESB adults (DE) and CELSPAC: SPECIMEn adults (CZ).

2.3.1.2. Potential presence using occurrence data in food commodities. For

the HBM datasets for which no dietary exposure data were available in van Klaveren et al. (2019) for the same country and same age category as in EFSA's considered population, a default potential presence was estimated using residue occurrence data in food commodities. Information of potential presence of substances in food commodities was retrieved from the same EFSA report (Table A.1.14 and Table A.2.14 from Annex A.1 and A.2 for CAG-NAN and CAG-NAM respectively) (van Klaveren et al., 2019). These values were derived from pesticide residue occurrence patterns obtained from the official national and EU monitoring programs for the period 2014–2016. Occurrence patterns were combinations of parent compounds assumed to co-occur within a given food sample, while all other parent compounds were assumed to be absent (van Klaveren et al., 2019). Based on these patterns, the potential presence of each parent compound contributing to a given metabolite was calculated for each parent compound–metabolite combination as:

$$PP_{k,m}(\%) = \frac{\sum_{j=1}^J x_{k,j}}{\sum_{k=1}^K \sum_{j=1}^J x_{k,j}} \times 100 \quad (b)$$

Where $PP_{k,m}$ is the potential presence of the metabolite m originating from parent compound k (%), $x_{k,j}$ is the potential presence value calculated by the MCRA toolbox for parent compound k in food commodity j , J is the total number of food commodities considered, and K is the total number of parent compound that are metabolised in metabolite m .

The obtained default potential presence value was then applied to the following HBM datasets and their respective population groups: 3xG, FLEHS 2 and 4 children (BE), ESTEBAN adults (FR), CELSPAC: SPECIMEn children (CZ), EXHES-ES (ES) pregnant women, SPECIMEN-NL children and adults (NL) and SLO-CRP children (SI).

2.3.2. Reverse dosimetry

Several methods can be used to estimate the external exposure of the parent compound based on HBM data (i.e., urinary concentrations of metabolites), including the steady-state equation (Sulc et al., 2022; Bartels et al., 2012; Tarazona et al., 2022) and the PBK modelling approach (Thépaut et al., 2024). Previous studies have shown that, for pyrethroids and chlorpyrifos, the steady-state equation yielded EDIs comparable to those obtained using PBK modelling (Thépaut et al., 2024, 2025). Consequently, in this study, we assumed that for low-persistent compounds in blood, such as pesticides included in the CAG-NAN and CAG-NAM, and under steady-state conditions, daily intake can be estimated using an equation based on the assumption of a linear relationship between the external exposure rate and the urinary excretion rates (i.e., fraction of urinary excretion (FUE)) of major metabolites.

Measured urinary concentrations of metabolites were converted into external exposures for each parent compound using a reverse dosimetry approach, based on equations adapted from Sulc et al. (2022). Equation (c) uses the concentration of a single urinary metabolite, preferably a parent compound-specific metabolite, to estimate the external daily exposure of the corresponding parent compound. Equation (d) integrates information from two urinary metabolites to estimate the external exposure to a single parent compound when several non-specific metabolites are measured in the HBM dataset. Equation (e) is a generalised version of equation (d), for a parent compound associated with more than two metabolites. In this study, this equation was applied to DAP metabolites, which may originate from several biotransformation pathways, including oxidative reactions such as de-arylation and esterase-mediated hydrolysis, sometimes occurring after an initial oxidative desulfurization step, prior to urinary excretion. To account for these different metabolic reactions, equation (d) or (e) is applied.

$$EDI_k = \frac{HBM-EL_m \times PP_{k,m} \times CE \times \frac{MW_k}{MW_m}}{BW \times FUE_m} \quad (c)$$

$$EDI_k = \frac{[HBM-EL_{m1} \times PP_{k,m1} + HBM-EL_{m2} \times PP_{k,m2} \times \frac{MW_{m1}}{MW_{m2}}] \times CE \times MW_k}{BW \times FUE_1 \times MW_{m1}} \quad (d)$$

$$EDI_k = \sum_i \left[HBM-EL_{m_i} \times \frac{MW_k}{MW_{m_i}} \times PP_{k,m_i} \right] \times \frac{CE}{BW \times FUE_m} \quad (e)$$

Where EDI_k is the estimated daily intake of parent compound k (in $\mu\text{g}/\text{kg}, \text{bw}/\text{d}$), $HBM-EL_{m_i}$ is the concentration of metabolite m_i in urine (in $\mu\text{g}/\text{g}$ of creatinine), PP_{k,m_i} is the potential presence of parent compound k associated with metabolite m_i (unitless), CE is the anthropometry- and gender-specific reference value for daily creatinine excretion in urine (g of creatinine/day), derived for children and adults (Remer et al., 2002; Forni Ogna et al., 2015), MW_k and MW_{m_i} are the molecular weights (g/mol) of parent compound k and metabolite m_i respectively, BW is the body weight of the individuals (kg), FUE_m represents the fraction of an absorbed parent compound that is excreted in urine as a given metabolite, expressed as the ratio of parent-compound intake to the amount of metabolite excreted (unitless parameter). Considering equation (e), FUE_{mr} refers to this fraction for the selected reference metabolite (m_r).

The use of the two equations (c) and (e) was combined for HBM datasets, where specific and non-specific metabolites were available. Molecular weights of parent compounds and metabolites were extracted from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and are presented in Supplementary Table S4. Human relevant FUE values were not available for all of the metabolites. Consequently, a default range of 0.05–0.95 was applied and an uncertainty analysis was conducted in MCRA, assuming an inverse uniform distribution. Daily urinary creatinine excretion rates were extrapolated from literature for children and adults (Remer et al., 2002; Forni Ogna et al., 2015) and are available in Supplementary Table S6.

2.4. Risk characterization

In line with EFSA's methodology, the total margin of exposure (MOET) was calculated for different population groups. Firstly, the margin of exposure (MOE) (equation (f)) was calculated for each parent compound as the ratio between the NOAEL for the common effect and the external exposure (i.e., EDI). The NOAELs for acute brain and/or erythrocyte AChE inhibition and for functional alteration of motor division, established in experimental animal studies and selected by EFSA, were used in this study to ensure consistency with EFSA's assessments (EFSA 2019b). NOAEL values for each parent compound included in the CAG-NAN and CAG-NAM are listed in Supplementary Tables S1 and S2. The reciprocals of the individual MOEs were summed to calculate the reciprocal of the MOET for the mixture (equation (g)). The resulting MOET value was then interpreted at population level and compared with an acceptable margin of exposure value (AMOE). In order to benchmark MOET results obtained in this assessment with those previously obtained by EFSA, a threshold of 100 was considered in this strategy as indicative of no appreciable health risk for the studied population (EFSA 2020). To identify the main risk drivers, the percentage contribution of each parent compound to the overall MOET was calculated within each dataset, focusing on individuals with MOET values below 100.

$$MOE_k = \frac{NOAEL_k}{EDI_k} \quad (f)$$

$$\frac{1}{MOET} = \sum_k \frac{1}{MOE_k} \quad (g)$$

Where MOE_k is the margin of exposure of parent compound k , $NOAEL_k$ is the no observed adverse effect level set as a toxicological reference point for the common effect, and EDI_k is the estimated daily intake of parent compound k , $MOET$ is the total margin of exposure of the mixture.

2.5. Risk assessment scenarios

Depending on the availability of HBM data in each dataset, and the underlying assumptions regarding metabolite formation (hereafter “metabolization hypothesis”), different scenarios were applied. For HBM datasets in which only specific metabolites were analysed (i.e., excluding DAP metabolites), the specific metabolite (SM) hypothesis was used and equation (c) was used to calculate the EDIs. Otherwise, for HBM datasets that include both specific and non-specific metabolites (i.e., DAP metabolites), the multiple metabolite (MM) hypothesis was applied. In this case, EDIs derived from specific metabolites were calculated using equation (c), while EDIs derived from non-specific metabolites were estimated using equation (d); thus, a combination of both equations was used. This resulted in three scenarios: “NAN-MM” (datasets including organophosphates and carbamates belonging to the CAG-NAN), “NAM-SM”, and “NAM-MM” (for datasets that included pesticides of the CAG-NAM and that relied on the SM or MM hypothesis, respectively). Table 2 summarises the scenarios applied to the different HBM datasets, together with the number of metabolites analysed, the number of parent compounds associated with these metabolites, and the population groups considered. The assumptions made for allocating metabolites to individual parent compound are presented in Supplementary Table S5.

2.6. Implementation in the Monte Carlo risk assessment software

HBM data treatment and MRA calculations were performed using the MCRA toolbox (<https://mcra.rivm.nl/mcra/#/>) (version 10.1). MCRA is a web-based platform designed to assess exposure to multiple chemicals and to perform MRAs (van der Voet et al., 2020). The software implements a two-dimensional Monte Carlo framework that allows the separate treatment of inter-individual variability in HBM data and uncertainty (related to factors such as left-censored data treatment and conversion factors) thanks to a two-loop framework. The first loop, called the inner loop, resamples individual HBM data measurements from the original database to account for population variability and to generate exposure distributions. The second loop, called the outer loop, quantifies the uncertainty associated with the results from the inner loop through bootstrapping of both the HBM data and the reverse dosimetry conversion factors, using 1000 iterations. The specific MCRA functionalities required to perform HBM-based MRA are described in (Engel et al., in prep). The results generated by MCRA were visualised with R (version 4.1.1) using *dplyr* and *ggplot2* packages (Wickham et al., 2014; Wickham 2016).

2.7. Comparison with mixture risk assessment based on dietary exposure

MOET obtained in this study was compared with those reported in EFSA’s previous assessment (van Klaveren et al., 2019). The 50th and 95th percentiles derived from the scenarios developed in this study were

compared with the corresponding percentiles reported by EFSA for countries and population groups that were comparable between the two assessments (Table 3). MOET estimates were compared with EFSA’s Tier I scenario, which consists of a model run with conservative assumptions. The percentage of individuals with a MOET below 100 and the main drivers of exposure at the 99.9th percentile under Tier I conditions were retrieved from Annexes C1 and C2 of the EFSA report (EFSA 2019a). The identified risk drivers identified in this study were then compared with EFSA’s tier I results, using the average contributions of raw primary commodities and active substances to the overall MOET (EFSA 2019a).

3. Results

3.1. Urinary concentrations

High variability was observed across HBM datasets regarding sampling periods, geographical coverage, measured biomarkers of exposure (i.e., metabolites), number of samples, urine type (morning, spot, 24-hour urine), detection and quantification limits, and the percentage of left-censored values (Table 4 for children and Table 5 for adults). Overall, the number of metabolites included in the assessment ranged from two (FLEHS 4 children (BE), CELSPAC: SPECIMEn children and adults (CZ)) to 11 (ESTEBAN children and adults (FR)). Carbofuran-phenol was the only N-methyl carbamates metabolite analysed and showed a high percentage of left-censored data. Regarding organophosphates, DAPs, 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY), and TCPy were the available metabolites. High percentages of left-censored TCPy data were observed in ESTEBAN children and adults (FR) compared to the other datasets, where the proportion ranged from 0% (SPECIMEn-NL children and adults (NL), 3xG (BE), EXHES-ES (ES)) to 36% (CELSPAC: SPECIMEn adults (CZ)). For DMTP and DEDTP, most of the values were left-censored. DMTP, DMP and DEP were among the most frequently quantified organophosphate biomarkers in ESB (DE) (Table 5). Pyrethroid metabolites considered in this study were 4-F-3-PBA, 3-PBA, *cis*-DBCA and ClF3CA. The proportion of left-censored values ranged from 60% (ESTEBAN children (FR)) to 100% (SPECIMEn-NL adults (NL)) for 4-F-3-PBA, and from 0% (ESTEBAN children and adults (FR), 3xG (BE), SPECIMEn-NL children (NL)) to 47% (CELSPAC: SPECIMEn adults (CZ)) for 3-PBA.

3.2. Potential presence of parent compounds

Across the available datasets, between 24 and 29 pesticides (out of 47) were retained for CAG-NAN, and between 11 and 43 pesticides (out of 100) for CAG-NAM. Probability of presence values of a given parent compound to be present as a residue in foods, considering the parent compound-metabolite combinations were calculated using equation a) or b) depending on the type of data used (exposure data versus occurrence data). The resulted potential presences values for CAG-NAN and -NAM are presented in Supplementary Table S7 and Table S8. Parent

Table 2

Proposed scenarios for acute mixture risk assessment of pesticides for CAG-NAN and CAG-NAM.

CAG	Metabolization hypothesis	Mixture composition	Number of parent compounds	Number of metabolites	Population groups	HBM studies
NAN	MM	Organophosphates, carbamates	27	8	Children	ESTEBAN (FR)
	MM	Organophosphates	24	6–7	Adults	FLEHS 2 (BE)
NAM	SM	Chlorpyrifos, chlorpyrifos-methyl, pyrethroids	12–16	2–5	Children	ESB (DE), FLEHS 2 (BE), FLEHS 3 (BE)
	MM		11–15	2–5	Adults	3xG (BE), CELSPAC: SPECIMEn (CZ), ESTEBAN (FR), SLO-CRP (SI), SPECIMEn-NL (NL)
	MM		12	2	Pregnant women	CELSPAC:SPECIMEn (CZ), ESTEBAN (FR), FLEHS 3 (BE), FLEHS 4 (BE), SPECIMEn-NL (NL)
						EXHES-ES (ES)

Abbreviations: BE: Belgium, CAG: cumulative assessment group, CZ: Czech Republic, DE: Germany, ES: Spain, FR: France, MM: multiple metabolites, NAM: functional alterations of motor division, NAN: acetylcholinesterase inhibition, NL: the Netherlands, SI: Slovenia, SM: single metabolite.

Table 3

Methodological hypotheses used for each dataset and population group in the MRA for CAG-NAN and CAG-NAM.

HBM surveys	Population group	CAG	Metabolization hypothesis	Population considered in EFSA assessment	Used potential presence
3xG	Children	NAM	SM	No	Occurrence data
CELSPAC-SPECIMEn	Children	NAM	SM	No	Occurrence data
	Adults			Yes	Dietary exposure
ESB	Adults	NAN	MM	Yes	Dietary exposure
		NAM			
ESTEBAN	Children	NAN	SM and MM	Yes	Dietary exposure
	Adults	NAM		No	Occurrence data
EXHES-ES	Adults	NAM	SM	No	Occurrence data
FLEHS 2	Children	NAN	MM	No	Occurrence data
	Adults			Yes	Dietary exposure
FLEHS 3	Adults	NAN NAM	MM	Yes	Dietary exposure
FLEHS 4	Children	NAM	MM	No	Occurrence data
SLO-CRP	Children	NAM	SM	No	Occurrence data
SPEICMEn-NL	Children	NAM	SM	No	Occurrence data
	Adults				

compounds with the highest potential presence values within CAG-NAN were azinphos-ethyl, chlorpyrifos, chlorpyrifos-methyl, dimethoate, omethoate, pirimiphos-methyl, tolclofos-methyl, and triazophos. For CAG-NAM, calculated potential presence values were the highest for azinphos-ethyl, chlorpyrifos, chlorpyrifos-methyl, dimethoate, ethion, lambda-cyhalothrin, pirimiphos-methyl, and triazophos. There was no probability of presence for oxydemeton-methyl and parathion-methyl. For the MRA they were not considered, resulting in a maximal number of parent compounds of 27 instead of 29 for CAG-NAN and 41 instead of 43 for CAG-NAM. Comparisons of calculated contributions using simulated exposure values against potential presence percentages obtained from occurrence pattern assumptions on food commodities data yielded different results. Using equation (b) for DEDTP, 77% was attributed to ethion, while using equation (a), more than 99% was attributed to azinphos-ethyl for CAG-NAN. For CAG-NAM the differences were not substantial.

3.3. Mixture risk assessment

The comparison across datasets of results presented from the current study has to be taken with caution due to overall heterogeneity in the available HBM datasets.

EDI values for each parent compound were calculated for CAG-NAN and CAG-NAM. The results are provided in [Supplementary Table S9](#). For example, the median EDI for chlorpyrifos for CAG-NAN in the MM scenario ranged from 0 to 0.11 µg/kg bw/day for adults and it was 0 µg/kg bw/day for ESTEBAN children (FR) and 0.115 µg/kg bw/day for FLEHS 2 children (BE). Regarding azinphos-ethyl, for adults, median EDI for was up to 0.25 µg/kg bw/day for CAG-NAN, and ranged from 0.01 to 0.166 µg/kg bw/day for CAG-NAM. For deltamethrin, for CAG-NAM using SM scenario the median EDI ranged from 0.01 to 0.08 µg/kg bw/day, while in the MM scenario the EDI for ESTEBAN dataset was of 0.06 µg/kg bw/day.

3.3.1. Mixture risk assessment for CAG-NAN

[Table 6](#) shows MOET estimates for the MM scenario for children and adults for CAG-NAN. This scenario considers multiple metabolites per parent compounds and includes up to 27 parent compounds. Three datasets (namely, ESTEBAN children and adults (FR) and FLEHS 2 children (BE)) had median MOET values higher than 100, however P90 and P95 MOET values were below 100 for all datasets except ESTEBAN for adults ([Fig. 1](#)). The mean contribution of the parent compounds to the MOET for individuals with a MOET below 100 identified azinphos-ethyl and triazophos as risk drivers for adults in FLEHS 2 and 3 (BE) and ESB (DE), and for children in ESTEBAN (FR) ([Fig. 2](#)). For the adult ESTEBAN (FR) population, carbofuran was the main risk driver with omethoate.

In the EFSA Tier I scenario for CAG-NAN, MOET values at median

exposure levels were 232 for children (France), 431 for adults (Belgium) and 394 for adults (Germany) (van [Klaveren et al., 2019](#)). In our study, MOET values at median exposure levels for ESTEBAN children (FR) and adults in FLEHS 2, 3 (BE) and ESB (DE) were 166, 64, 27 and 72, respectively. The percentage of individuals with a MOET below 100 in this study ranged from 58 to 97%, which is higher compared to the EFSA dietary assessment. Risk drivers for CAG-NAN in dietary risk assessment were chlorpyrifos, triazophos and omethoate, with dichlorvos, carbofuran and, formetanate for the selected populations. In the current study, for CAG-NAN, the risk drivers also include triazophos, carbofuran and omethoate.

3.3.2. Mixture risk assessment for CAG-NAM

For CAG-NAM, in the SM scenario, which considers specific metabolites and includes 12 to 16 parent compounds, MOET values at the 50th, 90th and 95th percentiles of exposure were higher than the AMOE of 100 in all datasets. MOET values at median exposure levels ranged between 3689 (3xG (BE)) and 18 460 (CELSPAC: SPECIMEn (CZ)) for children, and from 5775 for FLEHS 3 (BE) to 220,000 for EXHES-ES (ES) for adults ([Table 7](#)). The percentage of individuals with MOET below 100 was 0% for children and adults, indicating no health concern in the studied populations. Consequently, the mean contribution of parent compounds to the MOET was not assessed for this scenario.

In NAM-MM scenario, adding up to 25 pesticides including several organophosphates to the 14 pesticides used in NAM-SM scenario, MOET estimates were overall lower than in the NAM-SM scenario. For children, ESTEBAN dataset was the only dataset used, with MOETs at 95th percentile of exposure reported for children aged 6 to 10 years. For adults, MOET values at the 95th percentile of exposure were between 9 (FLEHS 3 (BE) and ESTEBAN (FR)) and 20 (ESB (DE)) ([Fig. 3](#)). The percentage of individuals with a MOET below 100 was lower for children (24% and 18.5% in ESTEBAN (FR)) than for adults (up to 93% for FLEHS 3 (BE)). The contribution of each parent compound to the MOET for individuals with MOET below the AMOE of 100 was similar for children and adults. The main risk drivers were organophosphates with azinphos-ethyl, triazophos, and ethion ([Fig. 4](#)).

In the EFSA Tier I scenario for CAG-NAM, for Belgium and Czech Republic adult population the MOET at the median exposure level was of 551 and 575, respectively. For children in France and the Netherlands, corresponding MOET values were 278 and 256 with a value of 97 at the 95th percentile of exposure (van [Klaveren et al., 2019](#)). The percentage of individuals with MOET below 100 in the NAM-SM scenario are aligned with EFSA Tier I results for the French (children), Dutch (children), Belgian (adults) and Czech (adults) datasets. In contrast, in the NAM-MM scenario, the corresponding percentages are overall higher in our study than in EFSA Tier I for the French (children), Belgian (adults) and German (adults) datasets. In the EFSA Tier II scenario, for exposure exceeding the 99.9th percentile, triazophos,

Table 4

Overview of limits of detection (LOD), limits of quantification (LOQ), proportions of left-censored data, and mean, median and 75th percentile urinary biomarker concentrations in µg/L of pesticide metabolites after left-censored data treatment across the HBM datasets for children.

Dataset (Country code)	Parameters	Organophosphates							N-methyl carbamates	Pyrethroids			
		TCPy	DMDTP	DEDTP	DETP	DMTP	DEP	DMP	Carbofuranphenol	4-F-3-PBA	3-PBA	Cis-DBCA	CIF3CA
3xG (BE)	LOD	—								—	—	—	—
	LOQ	0.1								0.1	0.1	0.1–0.8	0.1
	Left-censored data (%)	0%								97%	0%	22%	49%
	Mean	1.53								0.06	1.2	1.1	0.24
	Median	1.2								0.05	1.8	0.61	0.10
	P75	1.74								0.07	2.8	1.5	0.18
CELSPAC: SPECIMEn (CZ)	LOD	0.14									0.04		
	LOQ	0.46									0.14		
	Left-censored data (%)	18%									14%		
	Mean	3.2									0.68		
	Median	2.9									0.42		
	P75	4.5									0.7		
ESTEBAN (FR)	LOD	0.02	0.3	0.3	0.3	0.3	0.3	0.8	1	0.005	0.005	0.005	
	LOQ	0.05	0.6	0.6	0.6	0.6	0.6	2	5	0.015	0.015	0.015	
	Left-censored data (%)	98	100	100	61	5	78	59	99	60	0	0	
	Mean	0.001	—	—	1.73	9.6	1.04	4.6	0.3	0.03	2.2	2.4	
	Median	0	—	—	0.39	6.2	0	0	0	0.08	1.3	1.4	
	P75	0	—	—	1.58	13.7	0.46	6.9	0	0.04	2.5	2.8	
FLEHS 2 (BE)	LOD		1	2	1	1	2	3					
	LOQ		—	—	—	—	—	—					
	Left-censored data (%)		93%	64%	81%	7%	50%	38%					
	Mean		0.5	0.12	0.4	6.3	2.8	5					
	Median		0	0	0	4.3	0.52	3.6					
	P75		0.9	0	0	7.2	3.6	6.3					
FLEHS 4 (BE)	LOD	0.3									0.03		
	LOQ	0.5									0.09		
	Left-censored data (%)	3%									3%		
	Mean	3.2									0.99		
	Median	2.6									0.56		
	P75	4									1		
SLO-CRP (SI)	LOD	0.02								0.02	0.02		
	LOQ	—								—	—		
	Left-censored data (%)	10%								68%	11%		
	Mean	1.1								0.08	1.15		
	Median	0.51								0	0.65		
	P75	1.13								0.1	1.17		
SPECIMEn-NL (NL)	LOD	0.01								0.03	0.03	0.05	0.03
	LOQ	0.1								0.1	0.1	0.1	0.1
	Left-censored data (%)	0%								97%	0%	11%	31%
	Mean	1.3								0.002	1.9	1.5	0.26
	Median	0.85								0	1.2	0.65	0.13
	P75	1.4								0	2.5	1.3	0.29

Abbreviations: 3-PBA: 3-phenoxybenzoic acid, 4-F-3-PBA: 4-fluoro-3-phenoxybenzoic acid, BE: Belgium, *cis*-DBCA: *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, CZ: Czech Republic, DE: Germany, DEDTP: Diethyldithiophosphate, DEP: Diethylphosphate, DETP: Diethylthiophosphate, DMDTP: dimethyl dithiophosphate, DMP: Dimethylphosphate, DMTP: Dimethylthiophosphate, ES: Spain, FR: France, LOD: Limit of detection, LOQ: Limit of quantification, N: Number of individuals included in the dataset, NL: the Netherlands, P75: 75th percentile, SI: Slovenia, TCPy: 3,5,6-trichloro-2-pyridinol.

deltamethrin and omethoate were identified as the risk drivers for children (France), adults (Belgium, Germany). In our study, triazophos and omethoate were likewise identified as the risk drivers in ESTEBAN children (FR) and adults (FLEHS 2 and 3 (BE) and ESB (DE)).

4. Discussion

This proof-of-concept study explored how HBM data can be applied in practice to perform MRA for pesticides, and compared the results obtained with MRA based on external (dietary) exposure data, as

Table 5

Overview of limits of detection (LOD), limits of quantification (LOQ), proportions of left-censored data, and mean, median and 75th percentile urinary biomarker concentrations in µg/L of pesticide metabolites after left-censored data treatment across the HBM datasets for adults.

Dataset (Country code)	Parameters	Organophosphates								N-methyl carbamates	Pyrethroids			
		TCPy	DMDTP	DEDTP	DETP	DMTP	DEP	DMP	DEAMPY	Carbofuranphenol	4-F-3- PBA	3- PBA	Cis- DBCA	CIF3CA
CELSPAC: SPECIMEN (CZ)	LOD	0.137										0.04		
	LOQ	0.456										0.136		
	Left- censored data (%)	36%										47%		
	Mean	1.85										0.23		
	Median	1.4										0.12		
	P75	2.88										0.29		
ESB (DE)	LOD	—	—	—	—	—	—	—			—	—	—	
	LOQ	0.47	0.15	0.03	0.3	0.3	0.3	0.3			0.1	0.1	0.1	
	Left- censored data (%)	23%	88%	99%	57%	0%	0%	0%			96%	14%	50%	
	Mean	1.76	0.27	0.028	0.79	5.49	2.06	2.25			0.1	0.5	0.25	
	Median	1.3	0.13	0.024	0.42	3.32	1.52	1.46			0.08	0.32	0.14	
	P75	2.28	0.19	0.031	0.8	5.42	2.66	2.37			0.11	0.49	0.26	
ESTEBAN (FR)	LOD	0.02	0.3	0.3	0.3	0.3	0.3	0.8		1	0.005	0.005	0.005	
	LOQ	0.05	0.6	0.6	0.6	0.6	0.6	2		5	0.015	0.015	0.015	
	Left- censored data (%)	97%	97%	100%	63%	17%	53%	64%		98%	73%	0%	1%	
	Mean	0.006	0.04	—	1.41	6.8	7.04	6.1		0.374	0.026	1.67	1.76	
	Median	0	0	—	0	2.89	0.36	0.82			0.008	0.97	0.91	
	P75	0	0	—	1.36	7.12	3.67	4.79		0	0.023	1.73	2.04	
EXHES-ES (ES)	LOD	0.02							0.017		0.019	0.018		
	LOQ	—							—		—	—		
	Left- censored data (%)	0%							0%		0%	0%		
	Mean	0.05							13.6		0.13	0.8		
	Median	0.02							6.4		0.02	0.02		
	P75	0.04							9.9		0.04	0.07		
FLEHS 2 (BE)	LOD		1	2	1	1	2	3						
	LOQ		—	—	—	—	—	—						
	Left- censored data (%)		61%	91%	72%	16%	47%	45%						
	Mean		0.74	0.3	0.7	8	3	5.4						
	Median		0	0	0	4.5	1.8	2.8						
	P75		1.14	0	0.8	8.3	3.9	7.2						
FLEHS 3 (BE)	LOD	—	—	—	—	—	—	—				—	—	
	LOQ	0.5	1	1	1	1	1	1				0.09	0.8	
	Left- censored data (%)	12%	97%	99.5%	71%	45%	30%	61%				3%	92%	
	Mean	4.6	1	1	1.75	5.7	4.6	3.6				1.2	1.08	
	Median	2.8	0.78	0.77	1.2	2.6	2.8	1.5				0.78	0.66	
	P75	5	1.3	1.2	2.2	5.5	5.1	3.6				1.3	1.08	
SPECIMEn-NL (NL)	LOD	0.01									0.03	0.03	0.05	0.03
	LOQ	0.1									0.1	0.1	0.1	0.1
	Left- censored data (%)	0%									100%	2%	19%	31%
	Mean	1									—	0.9	0.6	0.23
	Median	0.88									—	0.74	0.36	0.13
	P75	1.24									—	1.1	0.57	0.22

Abbreviations: LOD: Limit of detection, LOQ: Limit of quantification, P75: 75th percentile, 3-PBA: 3-phenoxybenzoic acid, 4-F-3-PBA: 4-fluoro-3-phenoxybenzoic acid, BE: Belgium, *cis*-DBCA: *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, CZ: Czech Republic, DE: Germany, DEAMPY: 2-diethylamino-6-methylpyrimidin-4-ol, DEDTP: Diethyldithiophosphate, DEP: Diethylphosphate, DETP: Diethylthiophosphate, DMDTP: dimethyl dithiophosphate, DMTP:

Dimethylthiophosphate, DMP; Dimethylphosphate, ES: Spain, FR: France, N: Number of individuals included in the dataset, NL: the Netherlands, SI: Slovenia, TCPy: 3,5,6-trichloro-2-pyridinol.

previously performed by EFSA (van Klaveren et al., 2019; EFSA 2019a). This work contributes to the broader research effort on the potential role of HBM in improving human health risk assessment (Crépet et al., submitted) and supporting the prioritisation of pesticide monitoring and potential needs for evidence-based risk management. Traditionally, chemical risk assessment has relied on occurrence data to estimate external exposure levels on a substance-by-substance and on a source-by-source basis. However, incorporating internal exposure data from HBM studies conducted across European countries offers the opportunity to improve traditional risk assessment by considering mixtures and inherently accounting for several sources and routes of exposure. The experience gained from this exercise highlights both the potential added value of HBM-based MRA and the need to further develop robust methodological approaches that make optimal use of available data and knowledge, while explicitly identifying and accounting for the associated uncertainties.

In this study, MRA was applied across 10 European HBM datasets. This MRA resulted in high percentages of individuals with MOET values below the AMOE for cases based on the MM metabolism hypothesis. Conversely, the SM metabolism hypothesis did not show MOET below the threshold of 100. Identified main risk drivers were azinphos-ethyl, triazophos, and carbofuran for the CAG-NAN, and azinphos-ethyl, triazophos and ethion for the CAG-NAM. These organophosphates have the lowest NOAELs among the selected parent compounds and are currently no longer approved for use in the EU under the EU Pesticides Regulation. Consequently, no additional regulatory action can be taken with respect to the authorisation or use of these active substances within the EU. However, under EU legislation on pesticide residues (Regulation (EC) No 396/2005), food commodities imported from countries outside the EU may legally contain residues of non-approved pesticides, provided that applicable maximum residue levels (MRLs), including import tolerances or the default MRL, are not exceeded. As such, the identification of these substances as risk drivers remains relevant for dietary exposure assessment and risk management in the context of imported foods. At European level, regulators are currently exploring the possibility to set MRLs for non-approved substances.

The occurrence dataset used in EFSA's dietary risk assessment covered all EU member states for the years 2014–2016, while the HBM data analysed in the present study originated from sampling periods ranging from 2008 to 2020. Over this timeframe, new evidence on the toxicity of several parent compounds has emerged, leading to the revision or non-renewal of numerous pesticide approvals under the EU Pesticides Regulation. Such regulatory decisions result from the mandatory renewal procedure established under Regulation (EC) No 1107/2009, which involves a comprehensive scientific risk assessment conducted by a designated Rapporteur Member State and an EFSA-coordinated peer-review, in consultation with all Member States. As a consequence, by 2016 only eight of the 47 pesticides included in the CAG-NAN were approved for use in PPP under Regulation (EC) No 1107/2009 (Regulation (EC) No 1107/2009). Nevertheless, some parent compounds not approved at the EU level may still have been used during part of the study period under specific regulatory derogations, such as essential-use provisions (e.g., triazophos and ethion (Regulation (EC) No 2076/2002)).

In addition, EFSA's risk driver definition is based on the average contribution of the raw primary commodities and active substances exceeding the 99.9th percentile of exposure. In this study, due to data privacy constraints and small sample sizes in some datasets, the 99.9th percentile of exposure for the MOET could not be calculated. Despite methodological differences, the contribution of active substances resulted in similar parent compounds identified: triazophos, omethoate, and carbofuran for CAG-NAN and triazophos and omethoate for CAG-NAM.

Differences between MOET values obtained from the HBM-based assessments and EFSA diet-based assessment can partly be explained by the fact that some active substances have several authorised uses, resulting in exposure pathways other than food consumption. Indeed, several parent compounds included in the CAG-NAM are also authorised for use in biocidal products, such as product type 18 (PT18: (insecticides, acaricides and products to control other arthropods), under the EU Biocidal Products Regulation (Regulation (EU) No 528/2012), which entered into force in 2013. Thus, important sources of exposure to pyrethroids may include the domestic use of insecticides, mosquito nets, or insect repellent sprays. Pyrethroids are also widely used in veterinary medicine, particularly in deltamethrin-impregnated dog collars. Consistent with this, a French study by Vanacker et al. (2020) showed that, when considering external exposure data, cypermethrin and deltamethrin had the highest simulated internal exposure values across nearly all percentiles, with oral and dermal routes identified as the main exposure routes. Pet ownership and the use of insecticides on pets were identified as strong predictors of pyrethroid exposure. Similarly, in the ESTEBAN HBM study, the use of insecticides was reported as one of the main determinants of high urine pyrethroid concentrations in children and adults (Tagne-Fotso et al., 2023). In addition to these sources, residential proximity to agricultural land may also contribute to non-dietary exposure to pesticides (De Troeyer et al., 2022).

Moreover, differences between the two assessments also arise from the identification of parent compounds. In dietary exposure assessments, EFSA used the residue definitions for monitoring to allocate parent compounds to the measured residues in food (van Klaveren et al., 2019), whereas in the current MRA analysis, urinary metabolites were used as input data. One potential underestimation in dietary risk assessment may stem from the residue definitions used and assumptions on occurrence patterns, as most assessments consider only residue definitions for monitoring which may exclude some metabolites relevant for risk assessment. These residue definitions differ depending on the scientific assessment of each compound's risk profile (EFSA, 2020). This uncertainty along other uncertainties has been discussed by EFSA in their risk characterization report (EFSA, 2020).

The use of HBM urinary levels for pesticide MRA comes with certain limitations. In this current analysis, non-specific metabolites had to be proportionally assigned to parent compounds based on the likelihood of their presence in food in order to derive EDIs. This study shows that, depending on the approach (occurrence or exposure data), the estimated potential presence of parent compounds could vary substantially, leading to substantial uncertainty in the reverse dosimetry approach taken. We assumed that diet was the main source of exposure to pesticides when estimating the potential presence of parent compounds for the use of HBM data. This assumption is based on observations from previous aggregate exposure assessments where it has been observed for most people from general population that diet was the main source of exposure for pyrethroids among the other sources and routes considered (Vanacker et al., 2020; Oates and Cohen, 2011; Baudry et al., 2023). Occurrence data from ESFA reports were obtained from the official control activities carried out in the EU Member States, Iceland and Norway. The occurrence data used to calculate potential presence values could be another source of uncertainty as they are not necessarily representative of the country for which each dataset was collected. Therefore, using a potential presence of pesticides estimated only from food may underestimate or overestimate the contribution of some parent compounds to the overall exposure. Further methodological developments are needed to reduce uncertainty in the allocation of urinary metabolites to parent compounds while preserving the advantage of HBM data in integrating all exposure routes and sources. One possible consideration could be the use of multi-source exposure models to consider other exposure pathways such as domestic or biocidal

Table 6
Estimates of the total margin of exposure (MOET) and their 95th uncertainty intervals at the 50th, 90th, 95th percentiles of the exposure distribution in the multiple metabolite (MM) scenario for CAG-NAN in children and adult population groups.

Dataset (Country code)	Number of metabolites	Number of parent compounds	Sampling period	N	Population	MOET P50 [P2.5-P97.5]	MOET P50 EFSA results Tier I *	MOET P90 [P2.5-P97.5]	MOET P95 [P2.5-P97.5]	MOET P95 EFSA results Tier I *	% MOET < 100 [P2.5-P97.5]	% MOET < 100: EFSA Tier I results (%) *	% MOET < 100: EFSA Tier II results *
ESTEBAN (FR)	8	27	2014–2016	101	Children	166 [55–384]	232 [228–239]	22 [8–45]	15 [5–31]	72 [70–74]	38% [23–59%]	9.94%	0.21%
FLEHS 2 (BE)	6	24	2008–2009	144	Children	159 [39–308]	n/a	33 [8–67]	21 [5–42]	n/a	36% [17–58%]	n/a	n/a
ESB (DE)	10	24	2015–2020	180	Adults	72 [24–134]	394 [390–397]	26 [9–50]	19 [6–38]	130 [128–132]	66% [37–98%]	2.05%	0.08%
ESTEBAN (FR)	8	27	2014–2016	752	Adults	2485 [1031–4248]	n/a	319 [147–578]	126 [34–247]	n/a	2.8% [0.9–8%]	n/a	n/a
FLEHS 2 (BE)	6	24	2008–2009	140	Adults	64 [20–122]	431 [420–442]	15 [5–29]	11 [3–20]	148 [142–153]	58% [45–70%]	1.3%	0.07%
FLEHS 3 (BE)	7	23	2014	184	Adults	27 [12–55]		11 [4–23]	8 [3–17]		97% [82–100%]		

*Extracted from van Klaveren et al. (2019).

Abbreviations: BE: Belgium, DE: Germany, FR: France, MOET: Total margin of exposure, N: number of individuals in the dataset, n/a: non-available in EFSA assessment.

applications. In addition, deriving HBM-GVs with specific exposure biomarkers or specific effect biomarkers would reduce the uncertainty associated with pesticides MRA.

Another source of uncertainty in the reverse dosimetry approach is the use of linear equations considering one single metabolite preferably specific (SM) or multiple non-specific metabolites (MM). Using the MM equation, we assumed that contributions of the different metabolites could be summed. However, as some metabolites refer to different moieties of the parent compound depending on the metabolic pathway, this assumption is a conservative approach. For example, DAPs metabolites are non-specific biomarkers that are formed following different metabolism steps. Hydrolysis of the parent compound first produces dialkyl-dithiophosphates (DMDTP/DEDTP) that is chemically unstable and that will at a later stage be metabolized in dialkyl-thiophosphates (DMTP/DETP), that are more frequently quantified in HBM surveys. To analyse the impact on MOET results of such hypothesis, we tested an alternative allocation in using SM equation instead of MM for DAP as example (Supplementary Table S10). Applying SM or MM scenario led to similar results with an overlap of uncertainty intervals between the obtained MOET and the percentage of individuals with MOET below the AMOE. Other hypotheses made on reverse dosimetry parameters can also bring uncertainty. Daily urinary creatinine excretion rates were extrapolated from two studies (Remer et al., 2002; Forni Onga et al., 2015). Adults' creatinine excretion values were derived from two Swiss cross-sectional studies, which may not by fully representative of the broader European population. Finally, the scarcity of urinary excretion factors in the literature required the use of an inverse uniform distribution in MCRA to estimate kinetic conversion factors within predefined lower and upper bounds, adding additional uncertainty to the exposure reconstruction. As a consequence, the resulting EDIs used in risk characterisation have to be taken with caution.

Some urinary metabolites can be present in the environment prior to human metabolism, such as DAPs, which are non-specific organophosphate metabolites. Several studies indicate that DAPs may be present in residential environments or foods (Sudakin and Stone, 2011; Tsuchiyama et al., 2022). Organophosphates, once applied on crops, can be directly metabolised by plants into DAPs before human ingestion (referred to as preformed DAPs). A study using duplicate diet samples found a positive association between dietary DAP intake and urinary DAP levels, suggesting that preformed DAPs are the primary source of urinary DAPs, rather than direct exposure to organophosphate pesticides themselves. Indeed, over 70% of urinary DAP metabolite concentrations may be attributable to exposure to the preformed metabolites in the environment (Barr et al., 2011). Therefore, the presence of DAPs in urine reflects not only recent exposure to organophosphates but also exposure to non-neurotoxic DAPs (Tsuchiyama et al., 2022). This could have resulted in an overestimation of the exposure to organophosphates. We tested the impact of considering preformed DAP dividing the urinary concentrations of DAPs by 50 or by 100. This led to a substantial increase of MOET estimates and lower risk (Supplementary Table S11).

Comparisons of results between datasets should be interpreted with caution given the differences in age ranges, geographic coverage, sampling periods (from 2008 to 2021), urine sampling methods, analytical methods, LODs and LOQs, the range of metabolites measured, and the methods used to calculate the potential presence of parent compounds. Here in this study, the available datasets were for countries from Central (Germany, Czech Republic), Southern (Spain and Slovenia) and Western (Belgium, France, The Netherlands) Europe. These countries differ in climate, crop types, agricultural intensity and area, and thus in pesticide use. Moreover, some HBM studies may not be representative of a country's overall exposure. No statistical adjustment was applied to ensure representativeness of a country or a region (e.g., use of sample weights). Furthermore, the current HBM-based assessment may overestimate or underestimate exposure due to treatment of left-censored data in cases with low quantification frequencies. Therefore, the obtained results must be interpreted with caution.

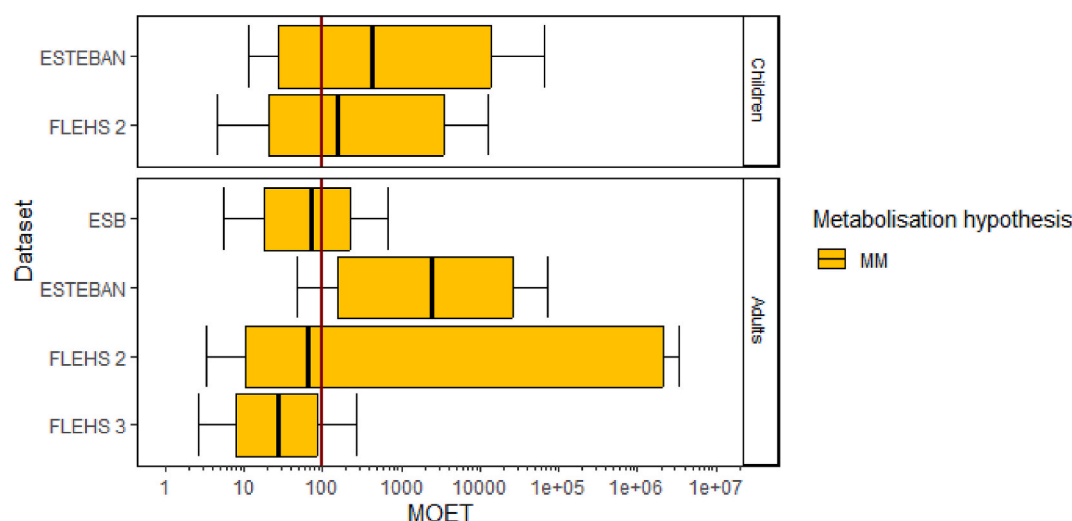


Fig. 1. MOET distribution per datasets for CAG-NAN. Boxplots display the distribution of MOET and associated uncertainty. The box limits represent the MOET variability across the population: high percentile (P97.5) – low percentile (P2.5) of the MOET, and the middle line, the median MOET. The whiskers show the uncertainty bounds of the distribution: the lower 2.5% limit of the 2.5th percentile, the upper 97.5% limit of the 97.5th percentile.

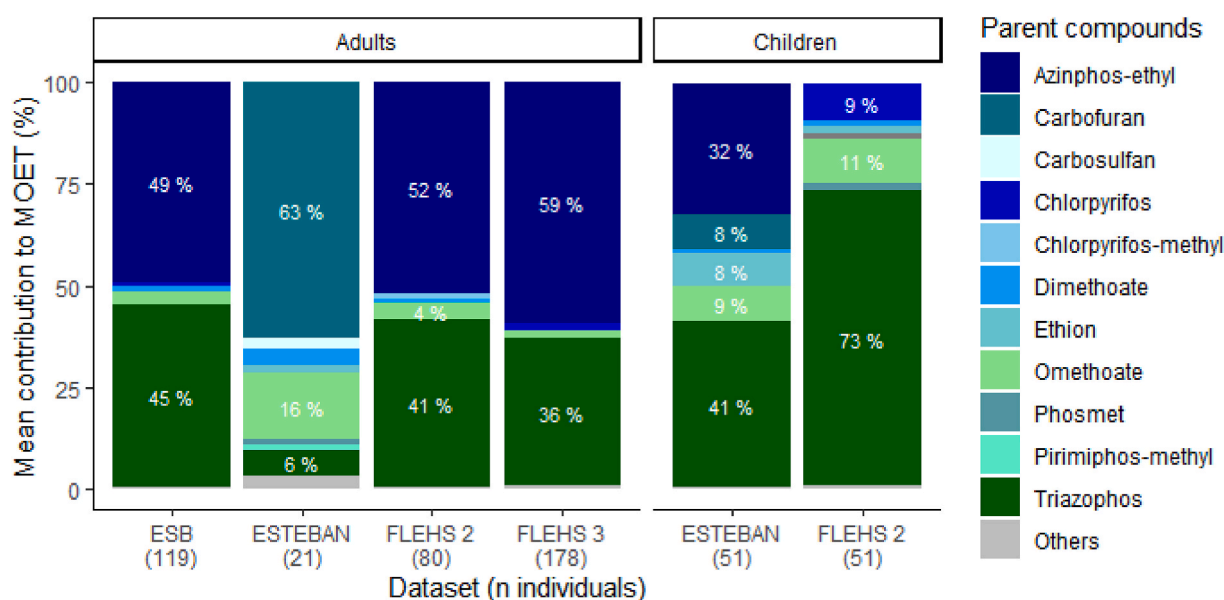


Fig. 2. Mean contribution of parent compounds to the MOET for individuals with MOET below the AMOE of 100 for CAG-NAN in children and adult population groups.

These limitations underscore the need for further research into more specific biomarkers of pesticide exposure and improved methods for assessing cumulative exposure to pesticides using HBM data. The absence of specific urinary exposure biomarkers currently prevents the establishment of internal toxicological values, i.e., HBM-GVs, which necessitates exposure reconstruction from HBM data to perform quantitative risk assessment (Carrier and Brunet, 1999; Timchalk, 2002; Poet et al., 2014; Zhao et al., 2022; Thépaut et al., 2024, 2025). In addition, few empirical FUEs are available in the literature for pesticides. Further research is needed in order to identify better urinary excretion of pesticides.

The available HBM datasets and their Supporting Information and study designs were not originally intended for pesticide risk assessment purposes, which introduces limitations in the accurate estimation of health risk assessment. To improve exposure attribution, harmonisation of HBM studies through specific experimental protocols should be developed considering the collection of the minimum amount of

information that apart from using the HBM data as a risk management tool, would allow the reliable risk assessment at population level, focused on the specific chemicals of interest and potential population needs such as sensitive populations. HBM questionnaires should harmonised between European countries on all potential sources of pesticide exposure, for instance, whether pet owners use pesticide-impregnated dog collars, which could aid in identifying sources of pyrethroid exposure. Future HBM studies in European populations should also consider collecting 24-h urine samples or multiple samples per participant. This would better characterise pesticide elimination and reduce the uncertainty related to sampling time and urinary excretion, as reflected by the FUE. Other improvements could include investigating analytical methods in other matrices for pesticide exposures, such as hair or breast milk, or combining data from the simultaneous sampling of several matrices. Furthermore, the harmonisation of data collection and analytical methods across studies is essential to ensure comparability at the European level (Esteban López et al., 2021; Govarts et al.,

Table 7

Estimates of the total margin of exposure (MOET) and their corresponding 95% uncertainty intervals at the 50th, 90th, and 95th percentiles of the exposure distribution for CAG-NAM for single metabolite and multiple metabolites scenarios in children and adult population groups.

Metabolization hypothesis	HBM dataset (country code)	Number of metabolites	Number of parent compounds	Sampling period	N	Population	MOET P50 [P2.5-P97.5]	MOET P50 EFSA results Tier I*	MOET P90 [P2.5-P97.5]	MOET P95 [P2.5-P97.5]	MOET P95 EFSA results Tier I*	% MOET < 100 [P2.5-P97.5]	% MOET < 100: EFSA Tier I results*	% MOET < 100: EFSA Tier II results*
SM	3xG (BE)	5	16	2019–2021	110	Children	3689 [1334–6982]		1406 [395–2718]	1060 [289–2122]		0%		
	CELSPAC: SPECIMEn (CZ)	2	12	2019–2020	85	Children	18,460 [5909–35060]		5620 [1701–11550]	3631 [1043–7855]		0%		
	ESTEBAN (FR)	3	14	2014–2016	101	Children	5974 [1764–12180]	278 [273–286]	1653 [462–3651]	1230 [310–2759]	97 [94–100]	0%	3.19%	0.09%
	FLEHS 4 (BE)	2	12	2017–2018	424	Children	15,840 [5363–29700]		5578 [1782–11240]	4030 [1256–8460]		0%		
	SLO-CRP (SI)	3	14	2018	149	Children	16,430 [4928–30380]		4988 [1431–9774]	2845 [751–6887]		0%		
	SPECIMEn-NL (NL)	5	16	2020	75	Children	11,410 [3428–22180]	575 [562–587]	3023 [729–8346]	1808 [339–4249]	199 [191–207]	0%	3.2%	0.07%
MM	ESTEBAN (FR)	11	39	2014–2016	101	Children	270 [127–514]	278 [273–286]	45 [19–85]	28 [10–54]	97 [94–100]	24% [12–45%]	3.19%	0.09%
SM	CELSPAC: SPECIMEn (CZ)	2	12	2019–2020	110	Adults	33,700 [11520–64260]		10,250 [3213–20940]	7115 [2012–15020]		0%	0.44%	0.09%
	ESTEBAN (FR)	11	14	2014–2016	752	Adults	5868 [1628–11250]		1769 [435–3473]	1231 [307–2410]		0% [0–0.66%]		
	EXHES-ES (ES)	3	15	2016	61	Pregnant women	220 000 [84400–385300]		2931 [797–7440]	1993 [564–4613]		0%		
	FLEHS 3 (BE)	3	12	2014	184	Adults	5775 [1865–10900]	551 [538–563]	2560 [777–5060]	1954 [571–4157]	211 [204–218]	0% [0–0.54%]	0.3%	0.01%
	SPECIMEn-NL (NL)	5	16	2020	101	Adults	16,560 [5040–30080]		6909 [1877–14320]	3934 [905–9824]		0%		
MM	ESB (DE)	10	35	2015–2020	180	Adults	78 [29–148]	494 [489–500]	28 [11–55]	20 [7–44]	181 [178–184]	62% [33–93%]	0.52%	0.015%
	ESTEBAN (FR)	11	39	2014–2016	752	Adults	150 [71–269]		16 [7–34]	9 [3–20]		41.6% [27.5–56%]		
	FLEHS 3 (BE)	9	34	2014	184	Adults	34 [15–62]	551 [538–563]	13 [5–25]	9 [3–19]	211 [204–218]	93% [78–99%]	0.3%	0.01%

*Values extracted from [van Klaveren et al. \(2019\)](#).

Abbreviations: N: number of individuals in the dataset; MOET: Total margin of exposure; SM: Single metabolite; MM: Multiple Metabolites, FR: France, DE: Germany, NL: The Netherlands, CZ: Czech Republic, SI: Slovenia, n/a: non-available in EFSA assessment.

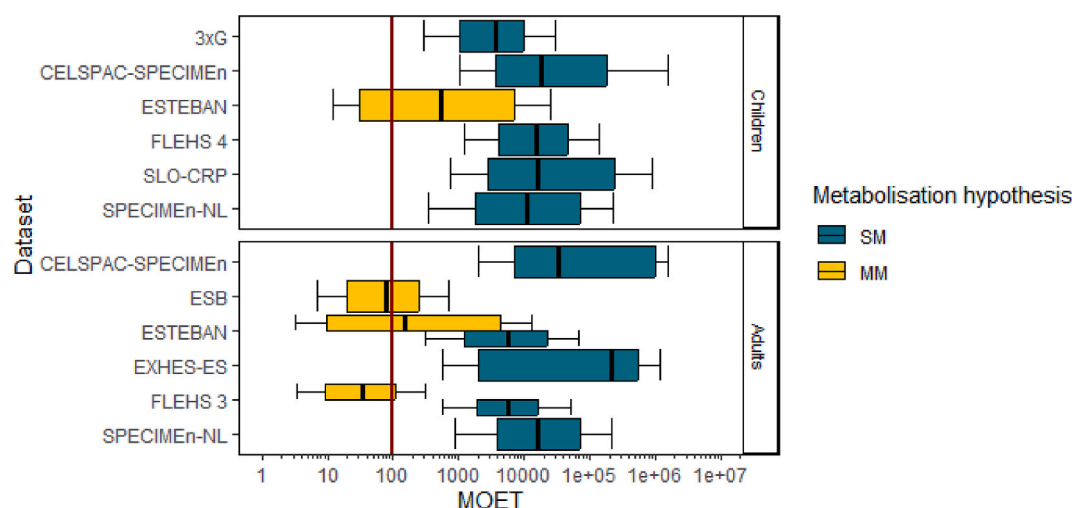


Fig. 3. MOET distribution per datasets for CAG-NAM. Boxplots display the distribution of MOET and associated uncertainty. The box limits represent the MOET variability across the population: high percentile (P97.5) – low percentile (P2.5) of the MOET, and the middle line, the median MOET. The whiskers show the uncertainty bounds of the distribution: the lower 2.5% limit of the 2.5th percentile, the upper 97.5% limit of the 97.5th percentile.

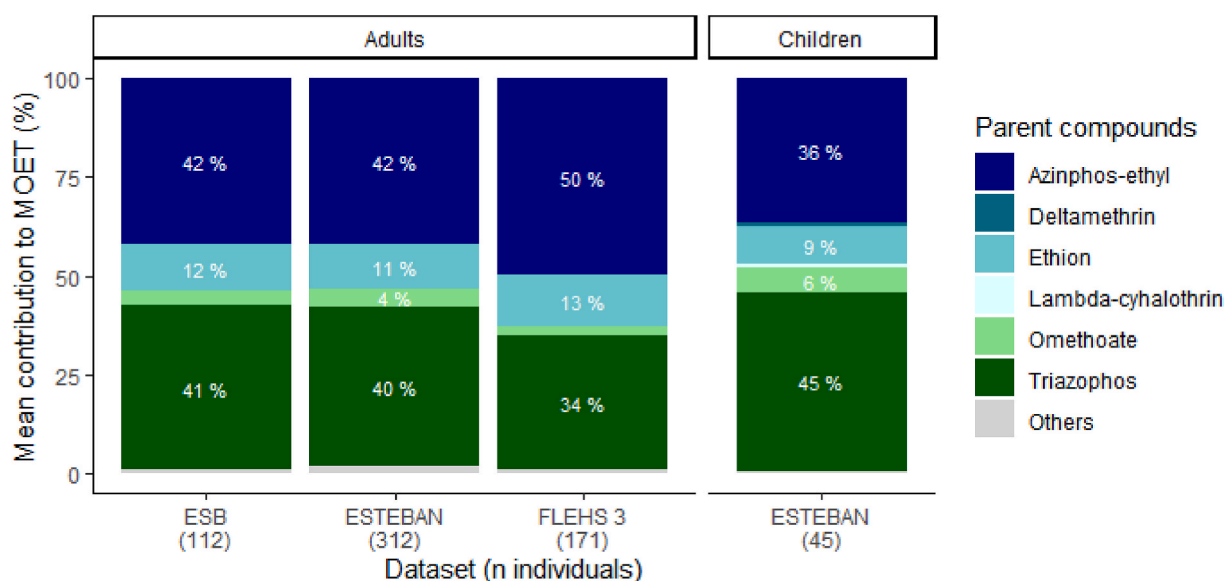


Fig. 4. Mean contribution of parent compounds to the MOET for individuals with MOET below the AMOE of 100 for CAG-NAM in children and adult population groups in the multiple metabolite (MM) scenario.

2023). This initiative is already taken in PARC with the launching of the PARC Aligned Studies. Establishing HBM-GVs for pesticides using specific exposure or effect biomarkers, based on reliable human toxicokinetic data, exposure data and exposure-effect estimates, would further improve health-risk characterisation using HBM data. Finally, PBK modelling and reverse dosimetry for relevant pesticide active substances could support the derivation of HBM-GVs by providing more accurate urinary excretion factors and precise information on substance-specific toxicokinetics across biological matrices.

5. Conclusion

Risk assessment for mixture of pesticides associated with acute neurotoxicity was performed using reverse dosimetry. External doses were estimated using urinary exposure biomarkers of pesticides from 10 European HBM studies, then risk characterization was performed using external toxicological values (NOAELs) for children and adults. Regarding CAG-NAN, the estimated MOETs were below the threshold of

100 in children and adults. For CAG-NAM, results showed no concern using the SM metabolization hypothesis for all populations however, using the MM metabolization hypothesis, the MOET were below 100 at high percentiles of the distribution. The cumulative exposure calculations in this study resulted in MOET values lower than those derived from dietary exposure estimates; nonetheless, the identified main risk drivers were largely consistent across approaches. Identified risk drivers were mostly phased-out organophosphate pesticides (azinphos-ethyl, ethion, triazophos). This proof-of-concept study demonstrates the potential of HBM data to complement traditional exposure assessments, improve human health risk assessment, support pesticide prioritisation in surveillance programmes, and inform risk management strategies. Several sources of uncertainty were identified, most notably the lack of specific biomarkers for many pesticides. Further advances in biomarker development, such as the identification of substance-specific exposure biomarkers, and development of fit for purpose experimental protocols for the collection of a minimum of the necessary information during HBM for the reduction of the sources of uncertainty in the HBM studies

are necessary to refine chemical MRA using HBM data.

CRedit authorship contribution statement

Philippe Palmont: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Conceptualization. **Madeline Carsique:** Writing – review & editing, Visualization, Validation, Resources, Formal analysis, Data curation, Conceptualization. **Karine Angeli:** Writing – review & editing, Validation, Resources, Methodology. **Hannah P. McKeon:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **Alexandra Mienné:** Writing – review & editing, Validation, Resources, Methodology. **Anastasia Spyropoulou:** Writing – review & editing, Validation, Resources, Methodology. **Adna Alilović:** Writing – review & editing, Validation, Resources, Formal analysis, Data curation. **Liesbeth Bruckers:** Writing – review & editing, Resources, Data curation. **Bianca Cox:** Writing – review & editing, Validation, Resources, Formal analysis, Data curation. **Pavel Čupr:** Writing – review & editing, Validation, Resources, Data curation. **Katleen Debrouwere:** Writing – review & editing, Resources, Data curation. **Deepika Deepika:** Writing – review & editing, Resources, Formal analysis, Data curation. **Clémence Fillol:** Writing – review & editing, Resources, Formal analysis, Data curation. **Eva Govarts:** Writing – review & editing, Resources, Data curation. **Tomáš Janoš:** Writing – review & editing, Validation, Resources, Formal analysis. **Marika Kolossa-Gehring:** Writing – review & editing, Resources, Formal analysis, Data curation. **Vikas Kumar:** Writing – review & editing, Resources, Formal analysis, Data curation. **Michelle Laeremans:** Writing – review & editing, Resources, Data curation. **Lucija Perharič:** Writing – review & editing, Resources, Formal analysis, Data curation. **Anja Remškar:** Writing – review & editing, Validation, Resources, Formal analysis, Data curation. **Janja Snoj Tratnik:** . **Libor Šulc:** Writing – review & editing, Data curation. **Nina Vogel:** Writing – review & editing, Validation, Resources, Formal analysis, Data curation. **Aude Ratier:** Writing – review & editing, Resources, Methodology. **Angelo Moretto:** Writing – review & editing, Methodology, Conceptualization. **Antonio F. Hernández-Jerez:** Writing – review & editing, Methodology, Conceptualization. **Waldo de Boer:** Writing – review & editing, Software, Resources, Methodology. **Johannes Kruisselbrink:** Writing – review & editing, Software, Resources, Methodology. **Jasper Engel:** Writing – review & editing, Software, Resources, Methodology. **Jacob van Klaveren:** Writing – review & editing, Supervision, Resources. **Kyriaki Machera:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Amélie Crépet:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Ethics declaration

Written informed consent to take part in the study and to publish the article has been obtained from all participants or their legal representatives. The privacy rights of participants have been observed.

Studies in human

This study was performed in compliance with relevant laws, regulatory frameworks and guidelines where the research took place.

This study relies on multiple human biomonitoring studies previously published:

- 3xG was approved by the Ethical committee of University Hospital Antwerp and University Antwerp (Approval No. A1058).
- CELSPAC-SPECIMEn was approved by the CELSPAC Ethics Committee (Approval No. ELPAC/EK/3/2019).

- ESB was approved by the Ethics committee of the Medical Association Westfalen-Lippe, the Medical Faculty of the University of Münster and (since 2012) by the ethical committee of the Medical Association of the Saarland (Approval No. Ha02/12).
- ESTEBAN was approved by the Ile-de-France Protection to person committee (Approval No. 2012-A00459-34).
- EXHES-ES was approved by the Ethical Committee of Clinical Research of the Sant Joan Hospital (Reus, Catalonia, Spain) (Approval No. 16-04-28/4aclaproy2).
- FLEHS 2 was approved by the Ethical Committee of the University of Antwerp (Approval No. A0809; A0809 addendum).
- FLEHS 3 was approved by the Ethical Committee of the University of the University hospital of Antwerp (Approval No. B300201318591; B300201316515; B300201419843).
- FLEHS 4 was approved by the Ethical committee of University Hospital Antwerp and University Antwerp (Approval No. B300201732753).
- SLO-CRP was approved by the National Medical Ethics Committee, Republic of Slovenia (NMEC) (Approval No. 0120-118/2017/3).
- SPECIMEn-NL was approved by the Ethical committee of METC, University Medical Centre Utrecht (Approval No. 19-513).

Fundings

This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014 with co-funding from the authors' organizations. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All co-authors report financial support was provided by Partnership for the assessment of risks from chemicals. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the EU Horizon 2020 program (grant No 857560, CETOCOEN Excellence), RECETOX RI (No LM2023069), AGEING-CZ (CZ.02.01.01/00/23_025/0008743), AZV CR No. NW25-04-00194 and MSCAfellow7_MUNI (No. CZ.02.01.01/00/22_010/0008854).

The CELSPAC - SPECIMEn study (Central European Longitudinal Studies of Parents and Children - Survey on PESticide Mixtures in Europe) and SPECIMEn-NL study were financed from HBM4EU EU Horizon 2020 Framework Project, HBM4EU, Grant number 733032.

The French human biomonitoring programme (ESTEBAN) is funded by the French ministries of Health and the Environment, and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail).

The EXHES-ES study was supported by funding from the European Union projects: HEALS (FP7-ENV-2013- 603946), Neurosome (H2020-MSCA-ITN-2017 SEP-210411486) and EDC-MET (H2020-HEALTH/0490-825762).

The funding of the German Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection is gratefully

acknowledged for ESB.

The 3xG study was commissioned and co-financed by NIRAS/ONDRAF (Belgian National Agency for Radioactive Waste and enriched Fissile Material), STORA (Study and Consultation Radioactive Waste Dessel) and MONA (Mols Overleg Nucleair Afval).

The FLEHS studies were conducted within the framework of the Flemish Center of Expertise on Environment and Health (FLEHS II, 2007–2011; FLEHS III, 2012–2015; FLEHS IV, 2016–2020). FLEHS II and III were funded by the Department of Economics, Science and Innovation, the Flemish Agency for Care and Health and the Department of Environment, Nature and Energy of the Flemish Government. The FLEHS IV study was funded by the Department of Environment & Spatial Development of the Flemish government.

SLO CRP is supported by the Chemicals Office of the Republic of Slovenia through the Jožef Stefan Institute program group P1-0143 (Slovenia), and a national project “Exposure of children and adolescents to selected chemicals through their habitat environment” (CRP-V3-1640, grant agreement No. C2715-16-634802).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2026.110531>.

Data availability

The authors do not have permission to share data.

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