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Population Exposure to Organic Contaminants and Human Biomonitoring

Norén, Erika

2023

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Norén, E. (2023). *Population Exposure to Organic Contaminants and Human Biomonitoring*. [Doctoral Thesis (compilation), Department of Laboratory Medicine]. Lund University, Faculty of Medicine.

Total number of authors:

1

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Population Exposure to Organic Contaminants and Human Biomonitoring

ERIKA NORÉN

OCCUPATIONAL AND ENVIRONMENTAL MEDICINE | LUND UNIVERSITY





We have measured internal exposure concentrations of environmental contaminants – that's the short version of how I usually answer what my thesis is about. The most common question that follows is *'Don't you think it's scary to know?'* but I think the opposite, that we wouldn't know anything about it, scares me more.

I hope this thesis can contribute to and be a part (even if it's just a very tiny part) of future work with human biomonitoring and its role in identifying and mitigating our exposure to environmental contaminants.



**FACULTY OF
MEDICINE**

Department of Laboratory Medicine
Division of Occupational and
Environmental Medicine

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2023:6
ISBN 978-91-8021-345-5
ISSN 1652-8220



Population Exposure to Organic Contaminants and Human Biomonitoring

Population Exposure to Organic Contaminants and Human Biomonitoring

Erika Norén



LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 20th of January at 09.15 in room 104, The Pufendorf Institute, Biskopsgatan 3

Faculty opponent
Thomas Göen

Organization LUND UNIVERSITY Division of Occupational and Environmental Medicine, Department of Laboratory Medicine Author: Erika Norén	Document name: DOCTORAL DISSERTATION	
	Date of issue: 2023-01-20	
	Sponsoring organization	
Title: Population Exposure to Organic Contaminants and Human Biomonitoring		
Abstract <p>We are surrounded by various chemicals, both naturally occurring and synthetically produced. Some, but not all, chemicals can be hazardous for human health following exposure, especially for vulnerable population groups including children, adolescents, and pregnant women. The increased use and production of chemicals have contributed positively to society in numerous ways, while also resulting in widespread contamination of the environment leading to unwanted human exposure. Knowledge of the magnitude of chemical exposure in populations and potential adverse health effects is insufficient. Human biomonitoring is one approach to estimating internal exposure by analysing defined exposure biomarkers in biological samples, such as blood or urine. It is a reliable measure of the total exposure of a substance from all routes of uptake and enables the assessment of simultaneous exposure to multiple compounds. Human biomonitoring can contribute with important data to improve these methods and the risk management of chemicals on a national and international level. However, human biomonitoring relies on toxicokinetic information of specific chemical compounds to ensure an appropriate sampling protocol that accurately captures the exposure, and for the interpretation of data within risk assessments.</p> <p>This thesis aims to apply human biomonitoring to investigate population exposure and temporal trends of organic contaminants in vulnerable population groups, i.e., adolescents, pregnant women, and infants, in southern Sweden. Furthermore, the included studies provide specific data on selected chemicals regarding toxicokinetics in humans, transplacental transfer efficiency, and whether analytical results of biological samples are affected after long-term storage. Exposure levels and trends were investigated for perfluoroalkyl substances (PFAS) and pesticides in adolescents by collecting blood and urine samples on five different occasions between years 2000 – 2017. We analysed 14 pesticide biomarkers in urine samples, where six were detected in more than 90% of the samples. Exposure to four substances, one of which is banned in Sweden and the EU, slightly increased over time whereas three decreased. The observed trends correspond with pesticide residues detected in domestic and imported cultivated food, and changes in use during the same period. Furthermore, we specifically investigated human elimination kinetics of glyphosate, the most commonly used herbicide globally. The results indicate that the urinary excretion corresponds to 1–6 % of the ingested dose, much less than the previously assumed urinary dose recovery based on animal studies. We assessed PFAS exposure in adolescents over time and prenatal exposure from transplacental transfer in a birth cohort with a wide range of PFAS exposures. In adolescents, PFOA, PFOS, and PFHxS were found in all serum samples but concentrations decreased over time. The decreasing trends of PFAS closely followed the stepwise international phase-out of these substances initiated in year 2002. Prenatal exposure to PFAS was estimated as ratios of umbilical cord serum and maternal serum in a cohort accidentally exposed to high levels through contaminated drinking water. The transfer ratios indicate that the relative transfer efficiency of PFAS is similar in highly exposed women, resulting in higher absolute fetal concentrations and an increased risk of potential health outcomes. The contribution of the thesis to the research field is that it reports novel knowledge of population trends in the exposure of PFAS and pesticides over 17 years, and considers how these trends align with key policy changes, important differences in elimination kinetics of glyphosate between animals and humans, analytical evidence supporting the stability and integrity of biological samples after long-term storage, and that the transplacental transfer of PFAS in highly exposed women result in high absolute fetal exposures. Additionally, the results address important aspects of sample collection procedures, interpretation of biomonitoring data, and the relevance of studying different population groups.</p>		
Keywords Human Biomonitoring, Population Exposure, Vulnerable Subgroups, Kinetics, Transplacental Transfer Efficiency, Exposure Biomarkers, Pesticides, Perfluoroalkyl Substances		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title: 1652-8220, Lund University, Faculty of Medicine Doctoral Dissertation Series 2023:6		ISBN: 978-91-8021-345-5
Recipient's notes	Number of pages 58	Price
	Security classification	

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Erika Norén



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Paper 4 © The Authors (manuscript unpublished)

Faculty of Medicine
Department of Laboratory Medicine
Division of Occupational and Environmental Medicine

ISBN 978-91-8021-345-5

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2023



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MADE IN SWEDEN 

Till Rasmus

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Populärvetenskaplig sammanfattning

Allt i vår omgivning består av olika kemikalier, både naturligt förekommande och syntetiskt framställda. Vissa är vi beroende av, som syret i luften, medan andra ämnen kan vara hälsofarliga om vi exponeras för dem, speciellt för känsliga grupper i befolkningen som exempelvis barn, ungdomar och gravida kvinnor. Inom miljömedicinen är syftet att undersöka och förebygga olika riskfaktorer i miljön som kan påverka människors hälsa negativt. Den kraftigt ökade användningen och framställningen av kemikalier har bidragit positivt till samhället på flera sätt genom exempelvis utvecklingen av medicinska behandlingar och förbättrad skadebekämpning som ökat avkastningen inom jordbruket. Den ökade användningen har dock medfört en ökad spridning av kemikalier i miljön vilket lett till en oönskad exponering av miljön och människor. Ända fram till 2000-talet har det varken funnits tillräcklig kunskap eller data om hur utbredd exponeringen av olika populationer är, eller hur exponeringen påverkar oss.

Ett tillvägagångssätt för att undersöka hur omfattande exponeringen i befolkningen är biologisk övervakning, så kallad *human biomonitorering*, som har använts för att undersöka yrkesexponering sedan 1930-talet. Det innebär att biologiska prover, vanligtvis urin eller blod, samlas in och analyseras för *biomarkörer*, i detta fall det kemiska ämnet vi kan ha exponerats för eller dess nedbrytningsprodukt. Detta är ett tillförlitligt mått på den totala exponeringen av ett ämne från alla upptagsvägar, och möjliggör analys av flera ämnen för att få en komplett bild av all samtidig exponering. Metoden förutsätter att lämpliga exponeringsbiomarkörer finns och att dessa går att analysera i biologiska prover med kvalitetssäkrade metoder. Exponeringen är ofta svår att definiera eller förutsäga i epidemiologiska populationsstudier eller modelleringar som är av relevans för att undersöka exponeringens koppling till hälsoeffekter eller kunna förutsäga potentiella exponeringsscenarior. Där är human biomonitorering ett viktigt komplement som bidrar med kunskap som delvis kan förbättra dessa metoder, och användas inom riskhantering av kemikalier på nationell och internationell nivå.

För att kunna tolka och använda data från human biomonitorering inom riskbedömningar behövs kunskap om de ämnen som analyseras, vad som händer när de absorberas i kroppen, hur de utsöndras och hur de bör analyseras. För att säkerställa att data reflekterar den faktiska exponeringen är det viktigt att ta hänsyn till detta i beslut om hur, när och vilka biologiska prover som samlas in.

I den här avhandlingen har human biomonitorering applicerats för att undersöka exponeringen av miljöföroreningar, med särskilt fokus på bekämpningsmedel och perfluorerade alkylsyror (PFAS) i sårbara populationsgrupper (ungdomar, gravida och nyfödda) och hur exponeringen förändrats över tid. De ingående studierna bidrar även med ny kunskap om detaljer för specifika kemikalier avseende metabolism och utsöndring hos människor, överföringen mellan mamma och foster under graviditet samt om analysresultaten av biologiska prover påverkas av lång tids förvaring i biobanker.

Exponeringsnivåer och trender undersöktes för bekämpningsmedel och PFAS i ungdomar i Skåne genom insamling av blod- och urinprover från totalt 1213 personer vid fem olika tillfällen mellan 2000 – 2017. Vi analyserade 14 biomarkörer för bekämpningsmedel i urinproverna och utav dessa detekterades sex i över 90% av proverna. Fyra ämnen, varav ett förbjudet i Sverige och nu även EU, ökade över tid medan tre ämnen minskade. De observerade trenderna hade tydliga kopplingar till resthalter detekterade i både inhemsk och importerad odlad mat samt förändringar av användningen eller inom lagstiftningen under samma tidsperiod. De mest använda perfluorerade ämnena PFOA, PFOS och PFHxS hittades i alla blodprover, men minskade över tid. Trenderna är tydligt kopplade till den internationella utfasning av dessa ämnen som skett stegvis genom både frivilliga och lagstadgade initiativ under aktuell tidsperiod.

I en experimentell exponeringsstudie undersökte vi utsöndringen av världens mest använda bekämpningsmedel glyfosat i urin hos människor. Tre volontärer gavs en känd dos av glyfosat och halterna i urin följdes i upp till 100 timmar efteråt. Resultaten tyder på att mängden glyfosat som utsöndras i urin hos människor är lägre än vad som tidigare uppskattats baserat på djurstudier, vilket har betydelse för tolkningen av mått på den inre exponeringen hos människor.

Vi undersökte exponeringen av PFAS hos nyfödda genom analys av blodprov från gravida kvinnor och navelsträngsprov från förlossningen i en populationsgrupp som oavsiktligt exponerats för höga halter från förorenat dricksvatten. Resultaten visar att överföringen är lika effektiv för dessa ämnen hos kvinnor med hög exponering, vilket resulterar i högre halter hos barnet vid födseln.

Resultaten bidrar med nya kunskap avseende exponering för mindre välstuderade bekämpningsmedel i allmänbefolkningen, hur trender i exponeringen för PFAS och bekämpningsmedel påverkats av policyförändringar under tidsperioden, att långtidsförvaring av biologiska prover inte påverkar analysresultat nämnvärt, att persistenta ämnen förs över lika effektivt hos högexponerade kvinnor till foster under graviditeten, samt viktiga skillnader i utsöndring av världens mest använda ogräsmedel hos djur och människor. Detta belyser även viktiga aspekter avseende provinsamling, tolkning av analysresultat samt relevans av att studera fler och olika populationsgrupper.

List of papers

- I. **Norén E**, Lindh C, Rylander L, Glynn A, Axelsson J, Littorin M, Faniband M, Larsson E, Nielsen C. Concentrations and temporal trends in pesticide biomarkers in urine of Swedish adolescents, 2000 – 2017. *Journal of Exposure Science & Environmental Epidemiology*, 2020, 30, 756–767
- II. Faniband M, **Norén E**, Littorin M, Lindh C. 2020. Human experimental exposure to glyphosate and biomonitoring of young Swedish adults. *International Journal of Hygiene and Environmental Health*, 2021, 231, 113657
- III. **Norén E**, Lindh C, Glynn A, Rylander L, Pineda D, Nielsen C. 2021. Temporal trends, 2000–2017, of Perfluoroalkyl Acid (PFAA) Concentrations in serum of Swedish Adolescents. *Environmental International*, 2021, 155, 106716
- IV. **Norén E**, Blomberg A, Lindh C, Pineda D, Jakobsson K, Nielsen C. 2022. Transplacental transfer of Perfluoroalkyl substances (PFASs) after long-term exposure to contaminated drinking water in the Ronneby MotherChild cohort. (*manuscript*)

Author's contribution to the papers

- I. Conceptualization, writing ethical application, recruitment, sample collection (year 2017), data curation, investigation, formal statistical analysis, writing original draft, reviewing and editing the draft, article submission, and reviewer correspondence.
- II. Data curation, recruitment, sample collection, reviewing and editing draft, article submission and reviewer correspondence.
- III. Conceptualization, writing ethical application, recruitment, and sample collection (year 2017), data curation, investigation, formal statistical analysis, writing original draft, reviewing and editing draft, article submission and journal correspondence.
- IV. Methodology, visualization, writing original draft, and reviewing and editing draft.

Abbreviations

ADI	Acceptable Daily Intake
AFFF	Aqueous Film Forming Foam
CEC	Commission of the European Communities
EC	European Commission
ECHA	European Chemical Agency
EU	European Union
EQUAS	External Quality Assurance Schemes
HBM	Human Biomonitoring
HBM4EU	Human Biomonitoring for Europe (international project/initiative)
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
PARC	Partnership for the Assessment of Risks from Chemicals
PFAS	Perfluoroalkyl substances
PFCA	Perfluorocarboxylic acids
PFSA	Perfluorosulfonic acids
REACH Chemicals	Registration, Evaluation, Authorisation and Registration of Chemicals
TTE	Transplacental Transfer Efficiency
UER	Urinary Excretion Rate
UNEP	United Nations Environmental Program

Introduction

Everything we touch, drink, eat and the air we breathe contains chemicals. Thereby, we are exposed to physical, biological, and chemical agents, including synthetic and naturally occurring compounds, in our everyday life. Some compounds, such as oxygen, are essential for our existence whereas exposure to other chemicals may be harmful or pose a risk to human health. The focus of environmental medicine is to study the interactions between such risk factors in our environment and human health (Howe 1980).

The large-scale use and application of chemicals have revolutionised numerous areas of society, improving our health and food security through increased pest control in agricultural food production and the development of new or more effective pharmaceuticals (Fama et al. 2022). At the same time, new or increased chemical exposure has had a negative impact on our environment and human health. In the 1960s, Rachel Carson (1962) raised concerns about the contamination of the environment through the wide application of pesticides, which increased the awareness of chemical management and initiated the modern environmental movement. Over the past four decades the amount and diversity of synthetic chemicals used, thereby also contaminating the environment, has increased at a rate incomparable to other ongoing global environmental processes of planetary change, including climate change (Bernhardt et al. 2017). The remaining question is how we can limit the negative effects associated with the chemicals that are already a part of our modern lifestyle and society.

There are legal frameworks in place to control the use of chemical compounds, that have been developed at national and international levels to mitigate human and environmental exposure. Still, knowledge about the toxicological profiles and properties of specific compounds, common exposure scenarios, and current exposure levels is needed to support policymaking. In the European Union, the production and use of chemicals are regulated through the Registration, Evaluation, Authorisation, and Restriction of Chemicals program (REACH), implemented in 2007. This framework only requires that chemical compounds used as pesticides or biocides must go through a complete risk assessment before being released on the market. For chemicals used at an industrial level, the industry needs to provide toxicological data on possible effects, usually obtained from experimental data on laboratory animals. Agencies and policymakers rely on this data to evaluate possible risks of the use and unintentional exposure to different chemicals, but what is often

missing and most difficult to predict is data on population exposure and potential risks to human health. Additionally, modeling is often used to predict possible exposure scenarios of new or previously not studied chemical compounds, but many synthesized chemical formulas do not behave as we expect when released into the environment. Human biomonitoring data can contribute to data that improve model predictions (Bevan et al. 2017).

Biological monitoring is a way to measure and quantify exposure from all sources by measuring the absorbed concentration inside the body using biological samples. By repeated measurements of the compound of interest or their metabolites in biological samples, human biomonitoring (HBM) can assess the exposure at the individual or population level and how it varies over time. According to United Nations Environment Program (2013), human biomonitoring has been emphasized as one of the primary health indicators to evaluate the effectiveness of sustainable management of chemicals. Today, human biomonitoring is considered crucial in the risk assessment process of chemical exposures to be used in combination with other tools, such as ambient monitoring (Jeddi et al. 2022; Tranfo, 2020).

Human biomonitoring

Human biomonitoring is the determination of concentrations of defined exposure biomarkers in biological samples as a measurement of internal exposure. A *biomarker* has several definitions but, in this context, refers to *exposure biomarkers* which is a chemical compound, or a degradation product/metabolite of that compound, measurable in biological samples. Biological samples, or *matrices*, commonly used for human biomonitoring purposes include urine, blood, breast milk, and hair. In contrast to ambient monitoring, which measures concentrations of chemical substances in environmental matrices such as air, water, and food, human biomonitoring considers all routes of uptake, includes all relevant exposure sources, and accounts for the kinetics of chemicals while measuring the internal exposure (Angerer et al. 2007). It is therefore a useful tool for exposure assessments within risk assessment, health prevention, and policy making. The data generated from human biomonitoring can be used in establishing reference ranges for specific chemicals, identifying highly exposed populations, studying time trends in exposure, and evaluating the effectiveness of policy measures taken (Bahadori et al. 2007). The measurement of biomarkers provides data and information that can be used as input in modeling which is the only available option to predict exposure before introducing chemical compounds on the market or to evaluate model predictions (Bevan et al. 2017). However, routine human biomonitoring in general populations is currently not anchored in legislation, as opposed to the monitoring of occupational exposure and external exposure sources (Jeddi et al. 2022). Analysis of internal exposure concentrations is a measure of the total exposure at a certain

point in time and cannot distinguish between the different sources of exposure, which is why ambient monitoring is still an important complement to being able to identify important sources of exposure to reduce the emissions (Angerer et al. 2007).

The concept of human biomonitoring was originally introduced in occupational medicine to ensure the safe conditions of exposed workers. The earliest examples of human biomonitoring in occupational settings are from the 1930s, when lead and benzene metabolites were quantified in blood and urine (Kehoe et al. 1933; Yant et al. 1936). However, measuring internal exposure requires analytical techniques that can detect low concentrations in biological matrices, which were not introduced and accessible for laboratories until the 1960s (Angerer et al. 2007). Lead was widely applied as an additive in gasoline but in the 1970s, evidence of its high toxicity was discovered (UNEP & OECD 1999). Human biomonitoring was then first applied in the context of public health to measure blood concentrations of lead in the general population in the USA in urban areas exposed to fuel emissions (Azar et al. 1975). In 1977, the Commission of the European Communities (CEC) also announced a directive requiring a screening program to be introduced in member states to assess lead exposure in the general population (Council directive 77/312/EEC 1977). Increased awareness of the toxic properties of lead combined with systematic measurements of lead exposure in general populations initiated the global reduction or phase-out of lead in gasoline (UNEP & OECD 1999).

In 1984, the concept of Human Biomonitoring was summarized through the following definition, published by Zielhuis (1984):

“a systematic continuous or repetitive activity for collection of biological samples for analysis of concentrations of pollutants, metabolites or specific non-adverse biological effect parameters for immediate application, with the objective to assess exposure and health risk to exposed subjects, comparing the data observed with reference level and – if necessary – leading to corrective actions”

Even though human biomonitoring has gained recognition since then and is increasingly applied as a tool for exposure assessment of hazardous substances, the definition remains pertinent.

The role of Human Biomonitoring

Continuous measurements of internal exposures can be used for different purposes, with the overall aim to create a chemically safe environment and mitigate exposure to hazardous substances in population groups or occupational settings (Ganzleben et al. 2017; Zidek et al. 2017). Analysis of biological samples enables measurements of both total compound-specific exposure levels but also simultaneous exposure to multiple compounds. Furthermore, it can show how the exposure varies with geographical area, age, and other factors relevant to identifying vulnerable groups

at risk. Repeated measurements over time make it possible to define emerging compounds but also to evaluate implemented restrictions of chemical use and its effect on chemical body burden at a population level.

Human biomonitoring can be applied to assess internal exposure in different target populations and across life stages (Jeddi et al. 2022). At a national and international level, it provides information on how exposure varies with i.e., geographic area, age, over time, or a combination of these (Vorkamp et al. 2021), data that can be used to define reference values for chemicals (Joas et al. 2012). Furthermore, it provides information on the proportion and characteristics of exposure and how it varies in different population groups, which makes it possible to identify more susceptible groups at risk (Gilles et al. 2021). It can also be used to evaluate if actions with bans or restrictions on chemicals have led to a decrease in internal exposure concentrations (Schulz et al. 2007; Wittsiepe et al. 2000). Reversed, it can also help to reveal elevated concentrations of internal exposure with time and be used as an early alarm for policymakers (Joas et al. 2012). Additionally, human biomonitoring can be applied to assess early biological effects and link biomarkers between exposure and effect to facilitate the identification of dose-response relationships (Louro et al. 2019).

Human biomonitoring has many strengths but also limitations, including its dependence on toxicological and metabolic data and that biomarker levels measured are very dynamic. However, modelling often has similar requirements of data input to support accurate exposure predictions. A combination of data from ambient monitoring, questionnaires, toxicology data from animal studies, and results from epidemiologic studies is ideal to facilitate the risk assessment process.

Key components for human biomonitoring

There are many important factors to consider when measuring human exposure to ensure that the internal exposure is captured as accurately as possible. For example, considering which biological matrix is most suitable for analysing a selected compound, what biomarker best reflects the exposure, and which intake or dose that corresponds to the measured concentrations in the biological samples. These decisions are based on previous research on specific compounds, both experimental and observational studies, to better understand the toxicology, kinetics, and biological mechanisms to evaluate possible risks of adverse health effects.

Critical factors to consider when assessing population exposure are generally related to the main components included in the human biomonitoring process, namely the choice of exposure biomarkers, the sample matrix, the protocol for sample collection and storage, and the analytical method used to quantify the exposure.

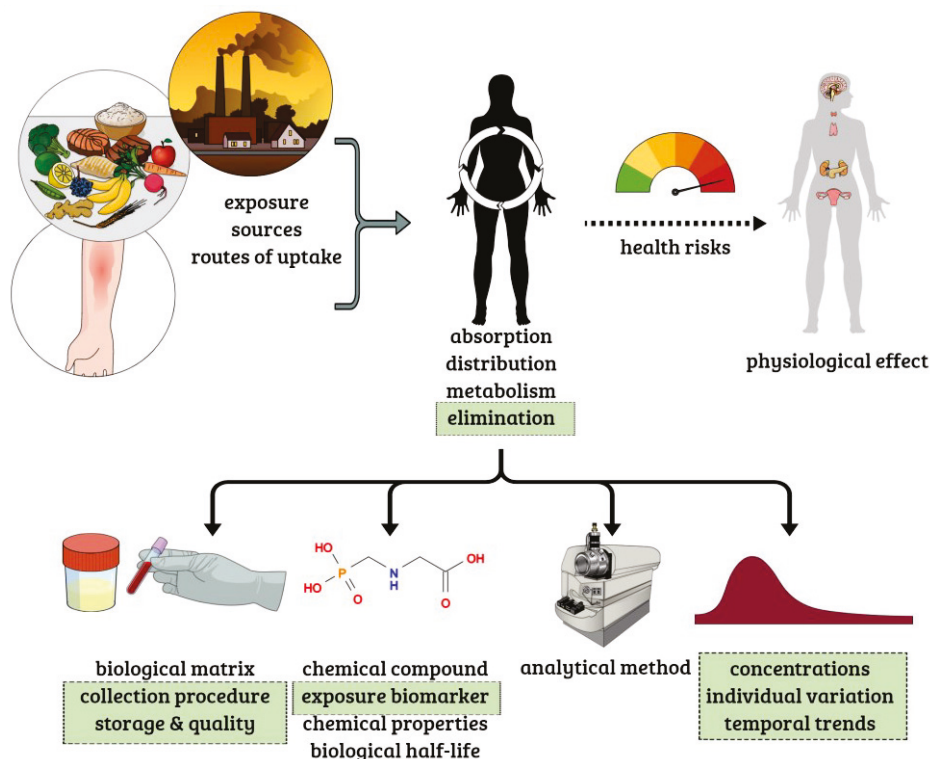


Figure 1. Schematic overview of key elements within human biomonitoring.

A summary of the concept of human biomonitoring and its important components. Key components addressed in this thesis are highlighted in green.

Exposure biomarkers

A selected exposure biomarker must be as *specific* as possible, where the measured concentrations exclusively are a direct consequence of and reflect an exogenous exposure (Hoet and Haufroid 1997; Vorkamp et al. 2021). Variations in dose should correlate with the measured exposure concentrations. Since there is always an intra-individual variability in biomarker concentrations it can be complicated to determine the exposure and it is, therefore, preferable if the *biological half-life* of a biomarker is not too short, as in a few hours or even less (Bevan et al. 2017). Additionally, biomarkers are more useful if their toxicokinetic properties, the biological fate of the parent compound or metabolites, and excretion profiles, are known (Bevan et al. 2017; Manno et al. 2010). Many chemicals are metabolized before they are excreted in the urine, and the parent compound might only be detected in low concentrations in urine samples. Therefore, relevant *metabolites* must be identified and used as biomarkers instead (Bevan et al. 2017).

Sample matrix

The *sample availability* can have practical implications, particularly if the most suitable matrix requires an invasive, time-consuming, or complicated collection procedure. Furthermore, maintained *compound stability and sample quality* must be guaranteed during sample collection and storage. For all biological sampling, a *validated sampling protocol* for the procedure of collection, transportation, processing, and storage is further needed. Storage conditions, such as temperature and duration, should be maintained during transport and storage prior to analysis and long-term storage in biobanks to avoid the sample quality being impaired. Variations in matrix composition also need to be considered in some cases, including the inter-individual variation in excretion rates (Hays et al. 2015). Hydration status mainly impacts urine dilution, and thereby the measured urinary concentrations, which can be adjusted for either the creatinine content or the specific gravity/density (Vorkamp et al. 2021).

Sampling strategy

Toxicokinetic data, including excretion profile and biological half-life, can help determine *the best time to sample* and extrapolate an estimate of the oral dose from internal exposure concentrations (Bevan et al. 2017). Furthermore, urine samples can be collected in different ways, for example as a 24-hour void urine sample or spot urine sample. Voided urine has been considered the standard procedure, but spot urine samples can be equally useful depending on the study objectives and the measured compound. The strategy chosen should be based on informed decision-making considering both toxicokinetic data and the elimination half-life of targeted chemicals along with the duration of exposure (Bevan et al. 2017). For human biomonitoring purposes, a repeated cross-sectional sampling strategy is preferred as it reflects a snapshot of the exposure at a specified time (Becker et al. 2014). A representative sample of a defined population group should be included, and a larger sample size can reduce the impact of both intra- and inter-individual variation, particularly for compounds with short biological half-lives (Angerer et al. 2006).

Analytical method

There is a long list of separate criteria for evaluating analytical methods in general, for example, as described by Vorkamp et al. (2021). The focus of this thesis is not on the general validation of analytical methods but on some important aspects of applying such methods in human biomonitoring.

An important part of verifying that analytical criteria are fulfilled, as well as the comparability of HMB results, is through both internal and external quality controls i.e., interlaboratory comparisons and certifications through analysis of reference materials (Göen et al. 2012; Nübler et al. 2022). For human biomonitoring purposes, sensitivity is also important, as in that detection limits are low enough to capture and quantify background exposure concentrations in the general population. At the

same time, the specificity of the measurements should be maintained. Interferences from endogenous compounds might also pose a problem as well as contamination of samples before, during, or after collection and analysis.

Interpretation of HBM population data

For human biomonitoring to be meaningful it is essential to interpret the data in the context of health risk assessment, which is often dependent on the available data for the specific compound or substance measured (Bevan et al. 2017). To make this possible, guidance values translated to measured internal concentrations are needed to improve and quantitatively understand the data in terms of the magnitude of exposure, health outcomes, or both. Current suggestions of guidance values to compare the measured concentrations with include either a value or range of the internal exposure representing the general background population or preferably a health-related reference value based on available toxicological or epidemiological data (Apel et al. 2020; Bevan et al. 2017; Gilles et al. 2021). The benefit of these values is that they can be used directly from measured internal concentrations without calculating the dose intake. A harmonized strategy for deriving such health-related values for internal exposure has been developed by the HBM4EU initiative and the work is in progress (Apel et al. 2020). These values are referred to as Human Biomonitoring Guidance Values (HBM-GVs) and are equivalent to HBM-I values developed by the German Human Biomonitoring Commission (Apel et al. 2020; Schümann et al. 2021). Although, sufficient toxicological-, epidemiological- and exposure data are for most substances usually not available.

Established Human Biomonitoring Programs

There are several examples of national biomonitoring programs that have been initiated. The National Health and Nutrition Examination Survey (NHANES), considered a pioneer program in human biomonitoring, was introduced in the 1960s in the United States to assess the health and nutritional status of the population through a nationally representative sample of individuals per year. After year 1999 it began measuring environmental parameters, including exposure concentrations of organic contaminants, in biological samples (CDC 2020; Johnson et al. 2013). The equivalent Canadian Health Measures Survey (CHMS) was launched in 2007 (Pollock et al. 2021).

Several examples exist in European countries as well, such as the German Environmental Surveys, GerES (Schulz et al. 2007), Czech Environmental Health Monitoring System (Cerna et al. 2012), French national biomonitoring program, ELFE (Dereumeauz et al. 2017), and PROBE in Italy (Alimonti. 2011) to mention a few. There is also a human monitoring program (HÄMI) in Sweden organized by the Swedish Environmental Protection Agency (Knudsen 2021). However, many

countries lack a nationwide program, and biomonitoring data is only collected within specific research projects.

In 2004, The European Commission recognized the need for a joint human biomonitoring initiative to increase the comparability of exposure levels across Europe in their Environmental and Health Action Plan 2004 (European Commission 2004). The first step towards an international program within the EU was taken in 2005 through some major studies exploring how such an initiative could be implemented (Schindler et al. 2014). In 2017, the common framework Human Biomonitoring for Europe (HBM4EU) was launched and ongoing until 2022, after which it continues as the Partnership for the Assessment of Risks from Chemicals (PARC) until year 2027 (Giles et al. 2021). The aim of both HBM4EU and PARC is to harmonize human biomonitoring data and increase the collaboration between research organizations and European health and safety agencies to provide science-based evidence for chemical policy development.

Exposure to environmental contaminants

Some, but not all, useful chemicals applied in the industry, agriculture, or consumer products possess toxic and potentially hazardous properties (EEA-JRC 2013; Prüss-Ustün et al. 2011). Individuals can be exposed to chemicals through ingestion, inhalation, dermal uptake, or a combination of exposure routes. We are usually exposed to several compounds simultaneously, but the health effects of combined exposure are poorly understood, and an approach for the cumulative risk needs to be further developed (Bil et al. 2022). It is not only the toxic properties of chemicals that are of concern for public health. High persistence and long biological half-lives are important for cumulative exposure, and most chemical compounds can be toxic if they exceed a certain concentration or dose (Cousins et al. 2020). Also, the susceptibility to exposure varies and certain population groups can be more sensitive to exposure. Overall, groups identified as more vulnerable to negative effects from chemical exposure include children, adolescents, pregnant women, and foetuses during sensitive windows of development (Kuipers and Mascolo 2017). Additionally, people with certain occupations or lower socioeconomic status can also be at increased risk (Kuipers and Mascolo 2017).

This thesis focuses on two major categories of organic contaminants with different chemical properties, *PFAS and agricultural pesticides*. Both PFAS and pesticides have been included on the list of prioritized compounds in HBM4EU, along with 16 other categories for which increased knowledge is needed to support policymaking. These prioritized groups were identified and selected based on concern for human health, previous evidence of exposure, and technical feasibility for analysis among others (Ougier et al. 2021).

Agricultural pesticides, also referred to as plant protection products, include herbicides, insecticides, fungicides, and plant growth regulators. They are designed to be toxic as they aim to control undesired targeted organisms, including insects, weeds, or fungi (Abreu-Villaça and Levin 2017). Because they are deliberately released into the environment, they also pose a risk to non-targeted organisms and human health (Manno et al. 2010). Many of the currently used pesticides have documented or suspected neurotoxic and/or endocrine disrupting properties that may interfere with neurologic or reproductive development (Abreu-Villaça and Levin 2017; Ntzani et al. 2013; Saillenfait et al. 2016; Saillenfait et al. 2015). As pesticides are applied as plant protection products they fall under Regulation (EC) 1107/2009 in the European Union, demanding an authorization process including prospective risk assessments prior to marketing. Established monitoring programs also measure residues of pesticides in food, which is the main exposure source for the general population (Bradman et al. 2015; Oates et al. 2006), and exposure concentrations in populations. Currently used pesticides have short biological half-lives and are metabolized and excreted in urine within a few days and are preferably measured in urine (Barr et al. 2005; Needham and Sexton 2000; Yusa et al. 2015). Prioritized categories include organophosphates and pyrethroids which are some of the most studied and commonly measured pesticides. Although, both data on exposure and toxicokinetics for glyphosate, the most used herbicide globally, are still surprisingly scarce (Connolly et al. 2020).

PFAS are manufactured organic compounds primarily used for their unique repelling properties, widely applied as surfactants in consumer products and packages. The strong carbon-fluorine bond makes them thermodynamically stable and resistant to degradation, ideal to use in materials to make them repellent to oil, dirt, and water (Buck et al. 2011; Kissa 2001). Their extensive use has resulted in ubiquitous presence in the environment and biota. PFAS have been associated with immunotoxic, neurotoxic, metabolic, and reproductive effects in humans (Chambers et al. 2021; Dalsager et al. 2021; Jiang et al. 2015; Schrenk et al. 2020; Spratlen et al. 2020). Because of this, the most widely used compounds have gradually been phased out of production since early 2000. Still, due to the persistence and accumulative potential of the ‘legacy PFAS’ PFOA, PFOS, and PFHxS, the exposure problem will remain for a long time. The general population is mainly exposed through diet/food (Richterová et al. 2022), where PFAS has been used in the coating of packages. In some local areas, elevated PFAS exposure concentrations have been discovered following contamination of the drinking water, originating from either industrial emissions or nearby military airfields using large quantities of aqueous film-forming foam (AFFF) in training activities. One example of a locally contaminated area is Ronneby in Sweden, where extremely elevated concentrations of AFFF-related PFAS were discovered in the drinking water following the use of firefighting foams at a nearby military airport (Xu et al., 2021). PFAS have long biological half-lives in humans, where quantified concentrations usually reflect long-term past exposure if the external exposure remains constant.

Aim

The overall aim of this thesis was to apply human biomonitoring to investigate population exposure and temporal trends of organic contaminants, including both short-lived and persistent compounds, in vulnerable population groups located in southern Sweden, and to provide compound-specific data on pesticides and PFAS highly relevant for improving the interpretation of human biomonitoring data.

Specific aims

- To assess exposure levels and temporal trends of biomarkers of agricultural pesticides analysed in urine samples of Swedish adolescents in the general population
- To assess exposure levels and temporal trends of perfluoroalkyl substances (PFAS) analysed in serum samples of Swedish adolescents in the general population
- To study the elimination kinetics of glyphosate, the most used herbicide globally, and its metabolite AMPA through human experimental exposure of three volunteers
- To assess transplacental transfer, and thereby prenatal exposure, of PFAS during pregnancy through matched maternal-cord samples from a cohort including highly exposed individuals in a contaminated area in Sweden
- To investigate if compound stability and sample integrity are affected by storage for up to 6 years

Materials and methods

Study design

Study I and III

A repeated cross-sectional study design was used to study temporal trends in exposure concentrations of pesticide biomarkers in spot urine samples and PFAS in serum samples from adolescents within the County of Skåne (within a 60 km radius of Malmö).

Study II

An experimental exposure study including three participants was used to investigate human elimination kinetics of the pesticide glyphosate and its metabolite AMPA through the administration of a known oral dose followed by sampling all voided urine for up to 100 hours. To further investigate the role of AMPA in relation to glyphosate, exposure concentrations of both substances were quantified in a cohort of adolescents with background exposure.

Study IV

A longitudinal birth-cohort with a wide range of PFAS exposures was used to estimate transplacental transfer efficiencies (TTE) of PFAS. Matched umbilical cord serum to maternal serum samples from pregnancy were used to calculate relative ratios of different PFAS and PFOS isomers in the overall cohort and stratified into three exposure categories to investigate the impact of the maternal exposure concentrations on TTE.

Study populations and sample collection

Study I, II & III

The study participants consisted of 1213 Swedish adolescents/young adults aged 17 to 21 years old. Recruitment of participants was performed in each of the years 2000, 2004, 2009, 2013, and 2017, as visualized in Figure 2. For the years 2000 – 2009, recruitment was performed through enrolment for military service in the county of Skåne, and therefore, only men aged 18 – 20 were included (the initial objective of

the sample collection was to assess semen quality parameters in relation to exposure to persistent compounds). After mandatory military service was abolished in Sweden in the year 2009, recruitment was relocated to secondary schools in Skåne.

In 2013, 13 years after the initial sample collection, the focus of the study was shifted to investigate temporal trends in exposure to environmental contaminants, and women were also included. A venous blood sample was collected by research nurses and each participant was instructed to collect a spot urine sample and distributed a paper mug and vial from our personnel.

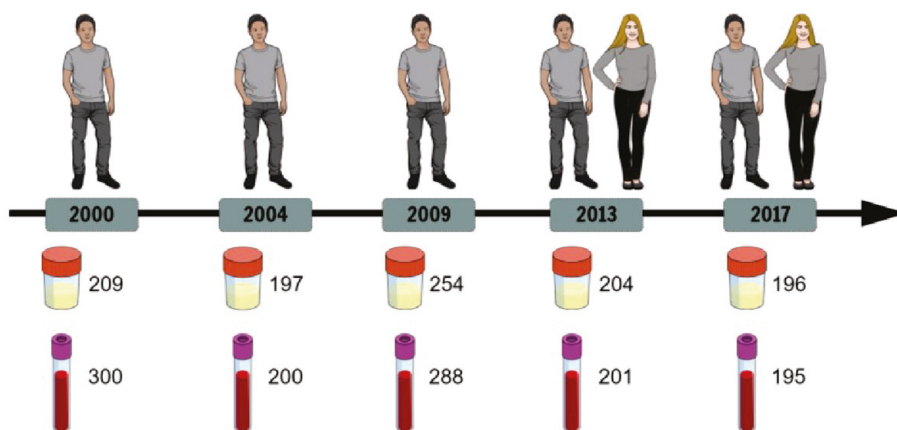


Figure 2. Visualisation of sample collection of adolescents

The number of urine and blood samples collected each year of recruitment of adolescents in Scania, aged 17 – 21 years from enrolment to military service (years 2000 – 2009) and secondary schools (years 2013 – 2017).

Study II

Three adults, one female and two males aged 42 – 73 years, gave written consent to volunteer in the human experimental exposure to glyphosate. After the volunteers were administered an oral dose of glyphosate, corresponding to 50% of the acceptable daily intake (ADI) calculated for each volunteer's body weight, all voided urine was collected for up to 100 hours as illustrated in Figure 3. The sampling time and urine volume of each sample were noted.

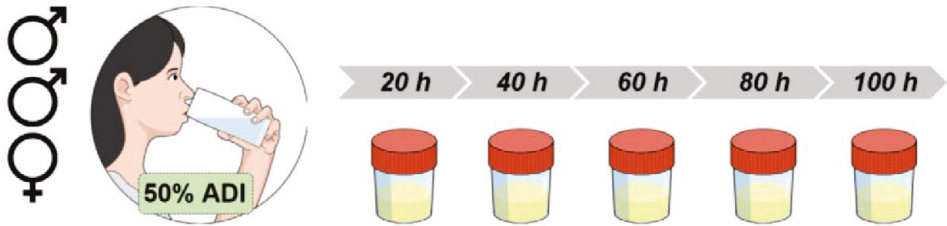


Figure 3. Illustration of the oral exposure experiment.

Three volunteers, two males, and one female were administered an oral dose of glyphosate corresponding to 50% of the acceptable daily intake (ADI: 0.3 mg/kg body weight/day) and all voided urine was collected for 100 hours.

Study IV

Study participants consisted of pregnant women in the Ronneby MotherChild cohort, a birth cohort consisting of 263 women, aged 18 – 43 years. The women were recruited during pregnancy between the years 2015 – 2020 in Ronneby and the nearby municipality Karlshamn. The cohort includes a wide range of PFAS body burdens, following long-term exposure to AFFF-related PFAS in drinking water in parts of the population. A serum sample was collected from the mother in the 2nd or 3rd trimester, and a second maternal serum sample and an umbilical cord serum sample were collected at delivery, as displayed in Figure 4. In total, 189 women provided matched maternal-cord serum samples.

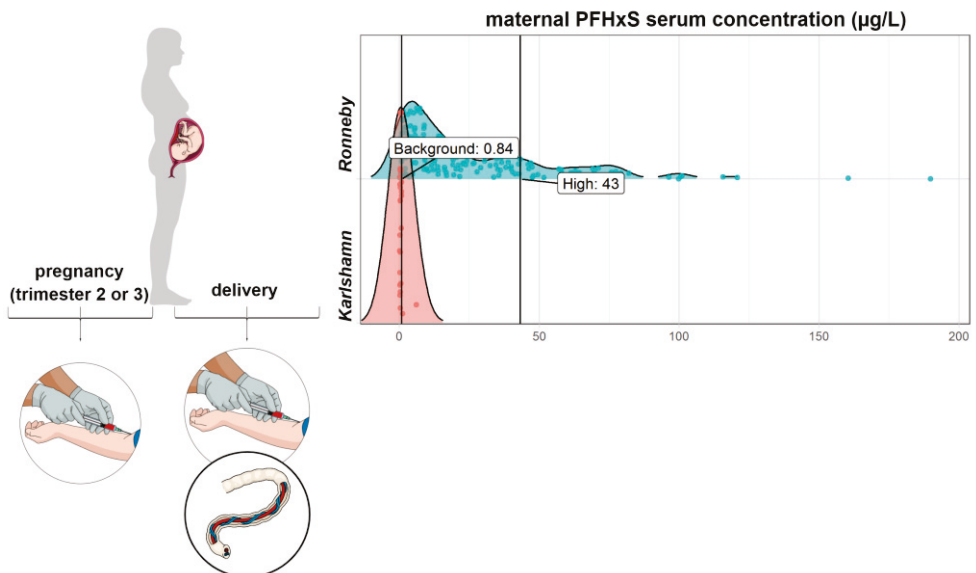


Figure 4. Illustration of recruitment and sampling protocol for the Ronneby MotherChild cohort.

Pregnant women were recruited in Ronneby or Karlshamn and maternal serum samples were collected during pregnancy and at delivery, where umbilical cord serum was also collected. The 189 mother-child pairs included in the current study provided matched maternal-cord serum and had a wide range of PFAS exposures.

Organic compounds and biomarkers

Two major categories of organic environmental contaminants were included in the thesis, pesticides, and PFAS. These are two of the 18 compound categories listed as priority substances in HBM4EU (European Environment Agency 2018). The specific compounds included, listed in Tables 1 and 2, have all been used globally during parts of or the entire period under study in this thesis. For several compounds, including some fungicides and growth regulators and AFFF-specific PFAS, available HBM data in general populations is limited.

Studies I & II

In study I, 16 biomarkers of pesticides listed in Table 1 were analysed in urine samples of young adults.

Table 1. List of pesticides and exposure biomarkers included in the thesis.

Pesticides and corresponding exposure biomarkers, listed as commonly used abbreviations, that were analysed in urine samples. Detailed chemical names are listed in studies I and II.

TYPE	GROUP	COMPOUND	BIOMARKER (ABBREVIATION)
Insecticides	Pyrethroids	permethrin	3-PBA
		cypermethrin	
		deltamethrin	
		resmethrin	
		fenvalerate	
		phenothrin	
		cyphenothrin	
		λ-cyhalothrin	
	cyfluthrin	4F-3-PBA	
bifenthrin	λ-cyhalothrin	CFCA	
		DCCA	
permethrin	cypermethrin	cyfluthrin	DCCA
chlorpyrifos-methyl			
Plant growth regulators	Quarternary ammonium	chlormequat	CCC
		mepiquat	MQ
Fungicides	Anilnopyrimidine	pyrimethanil	OH-PYM
	Benzimidazole	thiabendazole	OH-TBZ
	Triazole	tebuconazole	OH-TEB
	Dithiocarbamate	mancozeb	ETU
		propineb	PTU
Herbicides	Phenoxyacid	2,4-D	2,4-D
		MCPA	MCPA
	Organophosphate	glyphosate	GLY
			AMPA

Studies III & IV

The PFAS in Table 2 were measured in maternal and cord serum samples in the Ronneby MotherChild cohort (IV). All except the specific PFOS isomers were also measured in serum samples of adolescents over time (III). All the compounds, except PFPeS, are listed as priority substances in HBM4EU.

Table 2. List of perfluoroalkyl substances studied in the thesis.

CATEGORY	COMPOUND	ABBREVIATION (CHAIN LENGTH)
Perfluorinated sulfonic acids (PFSAs)	perfluorobutane sulfonic acid	PFBS (C4)
	perfluoropentane sulfonic acid	PFPeS (C5)
	perfluorohexane sulfonic acid	PFHxS (C6)
	perfluoroheptane sulfonic acid	PFHpS (C7)
	perfluorooctane sulfonic acid	PFOS, total (C8)
PFOS isomers	linear perfluorooctane sulfonic acid	n-PFOS (C8)
	perfluoro-1-methylheptanesulfonate	1m-PFOS (C8)
	perfluoro-2/6-methylheptanesulfonate	2/6m-PFOS (C8)
	perfluoro-3/4/5-methylheptanesulfonate	3/4/5m-PFOS (C8)
Perfluorinated carboxylic acids (PFCAs)	perfluorohexanoic acid	PFHxA (C6)
	perfluoroheptanoic acid	PFHpA (C7)
	perfluorooctanoic acid	PFOA (C8)
	perfluorononanoic acid	PFNA (C9)
	perfluorodecanoic acid	PFDA (C10)
	perfluoroundecanoic acid	PFUnDA (C11)
	perfluorododecanoic acid	PFDoDA (C12)

Chemical analysis

All biomarkers of PFAS and pesticides measured in urine or serum samples in the separate studies in this thesis were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS; QTRAP 5500 and 6500+: AB Sciex, Framingham, MA, USA) at the Division of Occupational and Environmental Medicine at Lund University. Samples were prepared in 96-well plate format. Quality control samples, chemical blanks (water), and calibration standards were included in each sample batch. All samples were analyzed between the years 2018 and 2021.

The limit of detection (LOD) and limit of quantification (LOQ) were defined from the standard deviation of concentrations corresponding to the peak at the same retention time as the analyte in the chemical blank samples.

The laboratory participates regularly in the German External Quality Assessment Scheme (G-EQUAS) inter-laboratory program for analyses of two pesticide biomarkers, TCPy and 3-PBA, and the compounds PFOS and PFOA. A visual summary of the laboratory performance is shown in Figure 5 with the quantified concentrations compared with G-EQUAS reference values in the provided samples,

for the years 2013 – 2022 of active participation in the interlaboratory program. Furthermore, the laboratory participated in HBM4EUs ICI/EQUAS exercises for analyses of the perfluoroalkyl substances PFHxS, PFOS, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA.

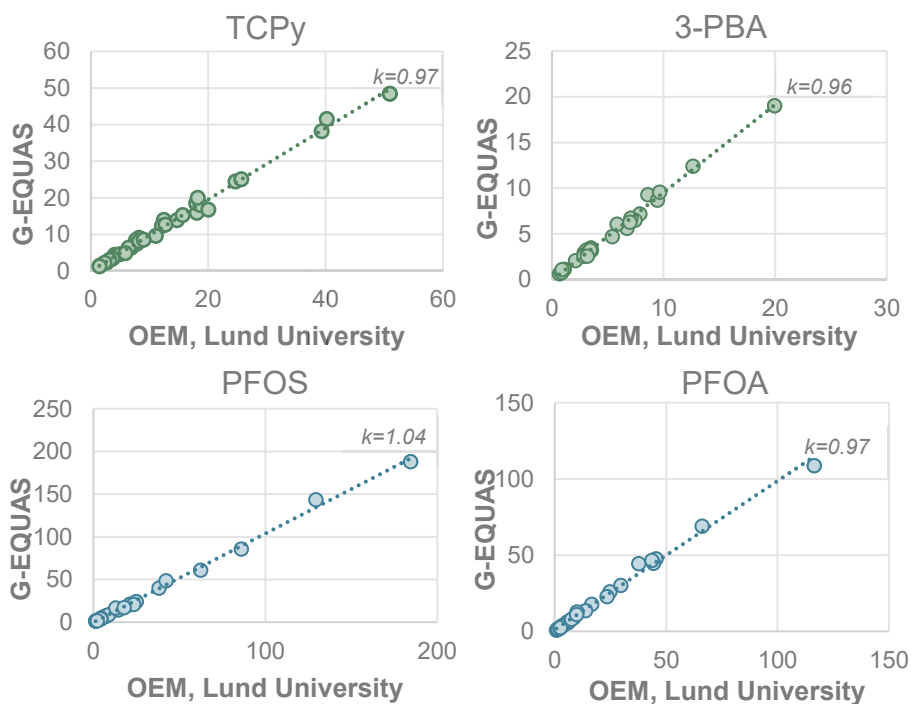


Figure 5. Visual summary of OEM laboratory performance.

A crude comparison of repeated analysis of concentrations of the pesticide biomarkers TCPy and 3-PBA in urine and the perfluoroalkyl substances PFOS and PFOA in serum. The figures show concentrations quantified by the laboratory at the Division of Occupational and Environmental Medicine (OEM) at Lund University plotted against the reference values in the samples provided by German External Quality Assessment Scheme (G-EQUAS), summarized for the interlaboratory program participation years 2013 – 2022, showing results close to unity.

Statistical analysis

Studies I and III

In both studies I and III, temporal trends in population exposure were assessed through linear regression of ln-transformed concentrations on calendar year, as a continuous variable. For compounds where a linear pattern was not observed, we explored quadratic terms centred around the mean. Measured concentrations <LOQ were included in the models without imputation. Model validation was conducted

through residual analysis using QQ-plots and a sensitivity analysis was conducted excluding female participants (in the sampling years 2013 and 2017).

In study I, compounds with at least 60%>LOD each sampling year were included in the statistical analyses, and in study III 70%>LOQ were included. Furthermore, observations with standardized residuals of >3 or <-3 were considered influential outliers and therefore excluded from the analysis in study III. Urine concentrations were adjusted for density and creatinine, where density-adjusted values were used in the main analysis. Positive values below LOD were used without imputation whereas negative values, caused by subtraction of chemical blanks, were substituted with the lowest positive concentrations <LOD.

In study III, the stability of PFAS in serum samples after up to 6 years of storage was assessed through Pearson correlation coefficient comparing the analysis performed in 2014 and a re-analysis in 2020. P-values <0.05 were considered statistically significant. The agreement between the two analyses was explored using Bland Altman plots.

Study II

Urinary excretion of glyphosate and AMPA were plotted as ln-transformed urinary concentrations against the time of sample collection (as mid-time points) from 0 up to 120 hours after intake of an oral dose of glyphosate, corresponding to 50% of ADI, for the three volunteers. Concentrations were plotted as unadjusted concentrations and adjusted for density, creatinine, or urinary excretion rate, separately. The elimination half-lives of glyphosate and AMPA in urine were estimated from the slope of the curve of ln-transformed concentrations of glyphosate and AMPA against the sampling time, separately for each volunteer.

Study IV

TTE were estimated by calculating the ratio of umbilical cord serum concentrations to maternal serum concentrations 1) in pregnancy, and 2) at delivery for the overall cohort. Furthermore, the cohort was categorized into three exposure categories (background, intermediate and high) based on maternal pregnancy serum concentrations of PFHxS, identified as a strong marker of AFFF exposure in Ronneby (Xu et al., 2021). We investigated if TTE varied with maternal exposure level for the AFFF-related PFAS where we observed a difference in the range of maternal concentrations between the categories. Because the blood volume increases in pregnancy, the impact of the gestational stage of sampling time of maternal blood was explored by comparing TTE when samples were collected in 2nd trimester, 3rd trimester, and at delivery.

Ethical considerations

The studies in this thesis include both invasive and non-invasive collection procedures of biological samples from humans and the handling of sensitive personal data. Further, it involves individuals or populations with background level exposure, deliberate oral exposure to a pesticide, and unintentional high long-term exposure to PFAS.

The studies were all separately approved by the regional ethics review board and complied with the International Ethical Guidelines for Health-related Research Involving Humans (CIOMS 2016). All participants involved in the studies signed informed consent before being allowed to participate and were informed that they could withdraw from the study at any time. Adolescents under the age of 18 years also provided a signature from a parent or guardian.

For the study population of adolescents, all data collected for the studies were anonymous since we did not keep records of names, addresses, or personal identification numbers. The collection of blood samples was performed according to the standardised procedure in healthcare. The only risk for participants in these studies was invasive blood sampling that in some cases can cause pain or bruising. They were all informed of the sampling procedure and possible discomfort prior to blood sampling. All participants could choose to have their results of urine and blood concentrations reported back, which could be seen as a benefit for participating. The contact details of the participants were deleted after reporting back the individual results.

In study II, we deliberately exposed three volunteers to a pesticide. The volunteers were given a single oral dose corresponding to 50% of the ADI, calculated based on the body weight of each volunteer to make sure the limit value was not exceeded. The current hazard classification of glyphosate, according to the Committee for Risk Assessment within the European Chemical Agency (ECHA), is 'causing serious eye damage and toxic to aquatic life', where ECHA concludes that further human toxicity classifications are not justified based on currently available knowledge. Since oral exposure to a pesticide does not provide any medical benefit, volunteers had to be healthy adults not under medication and not defined as in a risk group i.e., pregnant or nursing women or children. Furthermore, the participants were under the observation of a physician during the experiment. All volunteers provided informed consent.

The cohort in study IV includes pregnant women that provided informed consent. However, the main risk for participants in this study is past exposure to PFAS from contaminated drinking water. Studying and reporting results from a highly exposed cohort where possible adverse effects of the exposure are unclear is a very sensitive situation that might cause unnecessary anxiety or stress for study participants or other individuals related to their exposure situation. Conclusions from such studies,

and how those are communicated, need to be carefully considered. At the same time, the increased knowledge about the exposure situation and TTE of these compounds has large societal benefits. To protect the integrity of study participants, all data collected in the project is stored on a secure server at Lund University with two-way authentication where access is only granted to the researchers involved in the project.

Main results

The results of each study are divided into two categories, where results related to exposure monitored in population subgroups within each study are presented in the category ‘Population Exposure’, whereas the results of each study that address and provide data relating to key components of human biomonitoring are presented in the category ‘Aspects of key components in human biomonitoring’.

Population exposure

Study I

Six out of 14 pesticide biomarkers were detected in more than 90% of urine samples in all sampling years, indicating a low but widespread exposure. The highest median concentrations were detected for the biomarker CCC of the growth regulator chlormequat but were decreasing over time. The biomarker TCPy for chlorpyrifos was found in the second highest median concentration and increased over time, with an observed tendency to decrease after year 2009.

Study II

Urinary concentrations of glyphosate and AMPA were detected above LOQ in 20% and 29% of the samples, respectively, in 197 Swedish adolescents sampled in year 2017. The maximum concentration was higher for glyphosate (3.39 µg/L) than AMPA (0.99 µg/L) and the moderate correlation ($\rho = 0.56$) indicates separate exposures.

Study III

Serum concentrations of PFAS indicated widespread exposure in Swedish adolescents. Legacy PFAS (PFOA, PFHxS, and PFOS) had the highest median concentrations but decreased over time.

Study IV

Quantified serum concentrations of PFAS were higher in maternal serum than in cord serum. The AFFF-related compounds PFOS and PFHxS were found in the highest concentrations in both maternal and cord serum. Linear PFOS was

predominant of the isomers measured. Serum concentrations of the compounds PFHxS, PFHpS, PFOS, and PFOA were found in a wide exposure range in the study population, whereas concentrations of PFNA, PFDA, and PFUnDA were low and comparable to other background exposed populations i.e., Swedish adolescents in study III, for the whole cohort. Marginally lower TTE of compounds including PFHxS and PFOS in highly exposed mothers imply higher absolute prenatal PFAS exposure concentrations.

Aspects of key components in human biomonitoring

Study I

The study includes human biomonitoring data of exposure concentrations and temporal trends for pesticides not commonly measured (i.e., growth regulators and fungicides) in human biological samples but detected in food items. We found widespread exposure to chlormequat, where the biomarker (CCC) for chlormequat was detected in the highest concentrations of all pesticide biomarkers measured but with a decreasing trend. Low but widespread exposure was observed for the biomarkers of fungicides pyrimethanil (OH-PYM) and tebuconazole (OH-TEB) which increased over time.

Study II

Results from the oral experimental exposure to glyphosate showed that the urinary excretion followed a two-phase excretion with an elimination half-life of 6–8 hours for the short phase and 18–33 h for the longer second phase. The recovery of the oral dose of glyphosate was 1–6 % in urine in the three volunteers, much lower than previously used data around 20% based on animal studies. The metabolite AMPA was found in 0.01–0.04% of the administered glyphosate dose.

Study III

Comparisons of PFAS concentrations from analysis before and after storage at –80°C for 6 years yielded very similar results for PFOS, PFOA, PFNA, PFDA, and PFUnDA. Lower correlations and wider limits of agreement were observed for very low concentrations.

Study IV

Results of estimated TTE in a cohort with a wide range of PFAS exposure showed that subgroups with intermediate and high maternal PFAS exposure concentrations were similar to estimates for the overall cohort. Higher rates were observed for women at background exposure concentrations.

Discussion

This thesis has applied human biomonitoring to determine population exposure to organic contaminants in southern Sweden and used it as a tool to identify trends in exposure over time to emerging and phased-out compounds. Population groups included consist of adolescents from the general population and pregnant women with high exposure originating from contaminated drinking water. Thus, the thesis covers exposure across different life stages in population groups where data is currently lacking. Assessment of temporal trends in exposure span a period of 17 years (2000 – 2017) during which major international policy changes in chemical risk management were implemented. Furthermore, it provides new insights into aspects of key components important in human biomonitoring for obtaining valid results, including establishing new compound-specific toxicokinetic results on elimination and TTE at different exposure levels, along with evaluation of compound stability and sample integrity after long-term storage. Additionally, these findings address important aspects of the sample collection procedures, interpretation of biomonitoring data, and the relevance of studying different population groups. Taken together, the results of the thesis contribute to, and advance, current knowledge of important components of population exposure, as well as highlight important methodological aspects that may facilitate the future use and interpretation of human biomonitoring data.

Population exposure

Predicting or characterising exposure scenarios and distributions in models or epidemiological studies is difficult (Bevan et al. 2017), and large-scale biomonitoring of exposure is not considered a feasible option. Nevertheless, human biomonitoring in subsets or smaller cohorts can provide sufficient information on the range and variation in exposure concentrations of different groups in the population that can be applied in other settings (Gilles et al. 2021).

In studies I and III, widespread population exposure was observed in the low-concentration range in adolescents over time. These measurements give a snapshot of the prevalent exposure in this population group at different points in time. The results indicate if the exposure has any direction over time, either a tendency of increase or a decrease suggesting a change in emission patterns.

Overall, the observed trends in exposure aligned with or could be connected to reported patterns of chemical use, legal restrictions, or concentrations of residues detected in domestic and imported food items. For example, the highest median concentrations of pesticides in Study I were observed for the biomarker (CCC) of the growth regulator chlormequat, commonly used in rye production in Sweden (Mårtensson 2019). A decreasing trend in internal exposure concentrations was observed in adolescents between years 2000 and 2017. Measurements of residues of chlormequat in Swedish rye by the National Food Agency reported a detection frequency of 60% in year 2013 but 41% in year 2018 (Jansson & Fohgelberg 2016), which could be one possible explanation related to the decrease in internal exposure concentrations. During the same period, other growth regulators have been approved for use and are likely substitutions of chlormequat (Mårtensson, 2019). Overall, decreasing exposure trends do not only signal reduced emissions but also indicate potential replacement and introduction of a new compound or compounds that might be relevant to identify and include in future biomonitoring.

Chlorpyrifos, measured as TCPy, is a well-studied insecticide that has never been permitted for agricultural use in Sweden. Even so, it was detected in 99-100% of the samples with slightly increasing concentrations over time. Interestingly, as opposed to other biomonitoring studies, we did not observe a decreasing trend of TCPy, despite the stepwise reduced health-based guidance values and maximum residue levels implemented in the EU from year 2012 up until a complete ban in year 2020 (Tarazona et al. 2022). Although, the concentrations in Swedish adolescents were far lower than observed in other populations, including the years prior to the drastic reduction in exposure concentrations reported after 2016 in Spain and Portugal (Tarazona et al. 2022). This could be explained by the fact that national restrictions on chlorpyrifos have never permitted its use for agricultural purposes in Sweden. For compounds with short biological half-lives, such as many currently used pesticides, restricting the use and residue levels allowed in food items can drastically reduce exposure concentrations (Nguyen et al. 2019). Future follow-ups of the adolescent cohort will confirm if this is the case for TCPy.

Among the pesticides measured in Study I, some growth regulators and fungicides were included that are not commonly measured in biomonitoring studies of general populations. However, as residues of these compounds have been detected in food items in Sweden, both domestic and imported, they are relevant for human biomonitoring to determine the extent of population exposure. Additionally, analyses of multiple biomarkers of compounds provide data and context of aggregate exposure, which is difficult to obtain or predict through other methods. Chemicals with similar properties might be additive in terms of biological effects. Although, sufficient tools to interpret the effects of additive exposures need further refinement and reduce the level of uncertainty (Sieke 2018).

The observed trends in PFAS exposure were consistent with actions to phase out the compounds on an international level, initiated by the voluntary phase-out of PFOS

by the main producer 3M in 2002 followed by restrictions on the use of several PFAS in the EU starting in 2009. A decreased body burden of compounds with long biological half-lives indicates a discontinued or reduced exposure, implying that restrictive actions have had the desired effect on internal exposure concentrations.

The German Environment Agency has established so-called HBM-II values for PFOA and PFOS, which are set as a concentration in human biological samples which indicate relevant health risks if exceeded (Schümann et al. 2021). Established HBM-II values for PFOA and PFOS in blood plasma were 10 µg/L and 20 µg/L, respectively. If comparing exposure concentrations in Swedish adolescents in year 2000 with a median of 21 µg/L, that means 50% of the individuals exceeded the PFOS value relevant for health risks. The derived values were based on selected endpoints, including developmental toxicity and immunotoxicity. For women of childbearing age, which are considered a prioritized and vulnerable subgroup, the reference values were set at 5 µg/L for PFOA and 10 µg/mL for PFOS. Pregnant women in the MotherChild cohort in Ronneby had median serum concentrations of PFOS around 11 µg/L, in the range of relevant concentrations that, according to current knowledge, cannot exclude an increased risk of health effects. The most important exposure sources of legacy PFAS, both for general populations and populations with local emission sources, have been cut or phased out and these concentrations will likely continue to decrease with time. Nevertheless, these persistent compounds are accumulated in the environment where they will remain for a long time and likely continue to cause exposure to some extent. This may support the argument that persistence and accumulation potential alone are indications that should support restricted use (Cousins et al. 2020). For persistent compounds with long biological half-lives, these reference values can be problematic when they are found to be exceeded, as it may take years for the chemical body burden to reach acceptable levels with no concern for health risks. In these scenarios, well thought through risk communication strategies should be considered, especially for situations where unintentional exposure from contamination has occurred. For the remaining compounds included in this thesis, established HBM-II or equivalent reference values based on biological sample concentrations are not yet available. Therefore, the ability to further interpret available human biomonitoring data is currently limited because measurements of internal exposure concentrations cannot be directly translated to a dose intake. Still, generating this type of knowledge in future studies gives an indication of common background exposure levels in general populations and vulnerable subgroups.

Glyphosate and its metabolite AMPA were analysed in 197 adolescents in 2017. Concentrations were overall low and the detection frequency of glyphosate and AMPA were only 20% and 29%, respectively, despite being the most used plant protection product in the world. Previous studies have also reported median concentrations below the method detection limit (Beukers et al. 2022; Connolly et al. 2020). The low detection rate is likely explained by the low recovery of

glyphosate in urine. Results from the volunteer study in this thesis, and results reported by Zoller et al. (2020) indicate a urinary dose recovery of around 1-6%. In comparison, the recovery of TCPy in urine from an oral dose of chlorpyrifos is on average around 70% (Tarazona et al. 2022). It remains unclear if the low recovery of glyphosate in urine implies that it is mainly excreted in feces, as suggested in animal studies (Anadón et al. 2009), or if it accumulates somewhere in the body. Further studies on toxicokinetics, including analyses of glyphosate concentration in blood samples, are needed to clarify the relevance of the measured exposure.

Key components of Human Biomonitoring addressed

Exposure biomarkers

For most compounds included, exposure biomarkers and analytical methods are well-established and quality assured. However, relevant exposure biomarkers of the herbicide glyphosate are still being discussed. In the oral exposure experiment in Study II, volunteers were exposed to an oral dose of glyphosate, where the compound formula administered was analysed for AMPA content, that was not detected above LOD. Results showed a similar elimination curve to that of glyphosate but in very low concentrations (0.01–0.04% of the oral dose). The correlation of glyphosate and AMPA concentrations detected in a population group of Swedish adolescents was only moderate, and lower than expected based on the overall similarity in elimination half-life and dose recovery. This would support the theory of separate exposure sources as indicated by other studies (Beukers et al. 2022). This situation is not limited to glyphosate because metabolites, commonly used as biomarkers, of contemporary pesticides, are often formed through degradation processes in the environment prior to exposure meaning that the quantified exposure represents simultaneous exposure to the parent compound and metabolite (Fama et al. 2022). For some pesticides, the toxicological profiles are similar for the parent compound and formed metabolites, and the measurement of the aggregated exposure remains relevant in health risk assessments (Fama et al. 2022).

Sample matrix, collection protocol, and storage

In study I, random spot urine samples were collected from all study participants. Concentrations in these samples give a glimpse of the cross-sectional exposure to a substance but are less reliable than repeated samplings and more prone to exposure misclassification compared to 24 h void urine samples. Voided samples can be used in reversed dosimetry calculations and are more representative to compare to

acceptable daily intake or other limit values. This is especially a limitation for chemicals with relatively short biological half-lives and low dose recovery in measurements, such as glyphosate (Beukers et al. 2022). Still, spot urine samples are more accessible and the impact of intra-individual variability for spot urine samples can be reduced through increased sample size (Bevan et al. 2017). Inter-individual variability is also affected by urinary dilution, which is usually handled through adjustments such as creatinine correction or specific gravity/density. Since creatinine concentrations can vary based on characteristics including, age, sex, and diet it is more prone to introduce bias when comparing exposure levels between defined population groups (Bevan et al. 2017; Pearson et al. 2009), which is why density adjustment was preferred in the studies in this thesis.

The time window of sampling is of relevance. In study IV, the transplacental transfer of PFAS was assessed, which is the only fetal exposure pathway. Measuring persistent compounds in serum samples usually reflects cumulative exposure, which is fairly constant over time. However, during pregnancy the maternal serum concentrations are diluted because of increased blood plasma volume. Therefore, the gestational period of sampling is crucial to get an adequate measure of early pregnancy exposure. Although it would clearly facilitate the comparison of results between studies, there is no standard protocol for the timing of maternal sampling in the assessment of TTE of environmental contaminants. Early pregnancy should be the gold standard, but it is more difficult to access in practice due to the healthcare routines, where the first antenatal visit occurs late in the first trimester (in Sweden).

Most samples are stored for some time before the sample preparation and analysis and long-term storage in biobanks is common for biological samples used in retrospective studies, such as human biomonitoring. In study III, re-analyses in year 2020 of the serum samples collected in year 2013 enabled the comparison with results from the initial analysis in year 2014. The results imply that the compounds remained stable, and that sample integrity was maintained over time. It also confirmed that the analytical method is robust and well-validated, despite being performed by different technicians on different occasions. Lower correlations and wider limits of agreement were observed at low concentrations, where analytical precision is a challenge, and for PFHxS for which problems with observed interferences have been recognized (Chan et al. 2009).

The stability of the short-lived pesticide biomarkers TCPy and 3-PBA and sample integrity after long-term storage have been examined in urine samples collected in another cohort (i.e., Gyllenhammar et al. 2017) by comparing the concentrations in the same samples before and after four years of storage in -20°C (samples analysed in years 2014 and 2019). These samples were analysed in the same laboratory at the Division of OEM using the same instrumentation but by different laboratory technicians. The method had minor modifications compared to the method described in Study I. The comparison in Figure 6 indicates that these short-lived

biomarkers remained stable as well, and that sample integrity is maintained for at least four years.

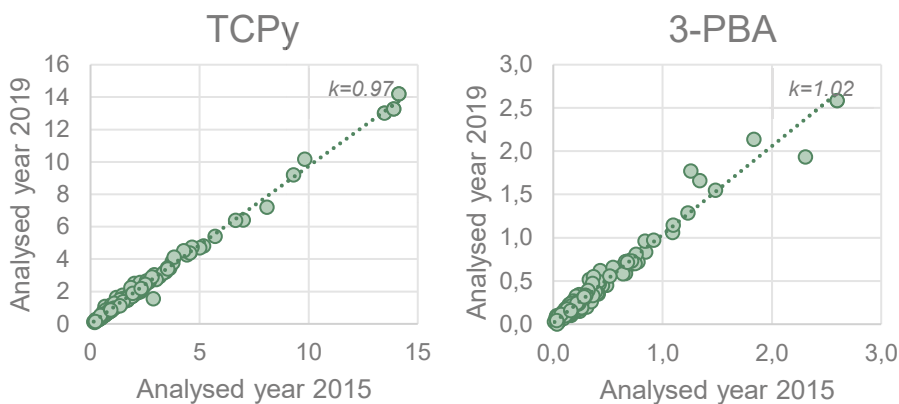


Figure 6. Quantified concentrations of biomarkers TCPy and 3-PBA in urine before and after 4 years of storage. A crude visual comparison of the quantified concentrations of TCPy and 3-PBA in urine samples (n=177) analysed in year 2015 plotted against concentrations in the same samples analysed in year 2019 using the same analytical method before and after up to four years of storage.

Toxicokinetic data

The assessment of human elimination kinetics and dose recovery of glyphosate in urine in Study II showed different results compared to results from animal studies. These results suggest that reversed dosimetry estimations in populations based on toxicokinetic data from animal studies underestimate the ingested dose (Connolly et al. 2020). The low recovery of glyphosate in urine is also important to bear in mind when assessing simultaneous exposure by measuring internal exposure concentrations, since these concentrations when compared do not represent the relative exposure of each compound due to differences in excretion and dose recovery.

Volunteer studies with exposure to specific compounds are not only useful for retrieving toxicokinetic data on metabolism and elimination in humans but can also facilitate the translation of internal exposure concentrations to a known administered dose and defined exposure route. Even when such studies include very few volunteers it gives an indication of detectable concentrations from a known exposure dose that helps to provide contextual data for the interpretation of human biomonitoring results (Bevan et al. 2017). A better understanding of the toxicokinetics for biomarkers of exposure will facilitate the interpretation of human biomonitoring data but human data is scarce and extrapolation from animal studies can be misleading.

Strengths and limitations

The thesis has several strengths. It builds on globally unique datasets with adolescents with repeated cross-sectional sampling for 17 years and pregnant women with a wide range of exposures, and it considers both methodological aspects and practical applications of HBM. Thus, the included research covers several important aspects and application areas of human biomonitoring, from sampling strategies to time trends. Furthermore, the thesis covers environmental contaminants with both short and long elimination half-lives and contextualizes the challenges involved in HBM of compounds with different chemical properties.

We have included some pesticides and PFAS not commonly measured or monitored in previous population-based studies in general populations. Pesticides biomarkers of fungicides and growth regulators have been measured in occupational settings but rarely in individuals from the general population. Additionally, some of the AFFF-related PFAS are rarely detected in general populations and therefore less studied. We have also provided exposure data from prioritized groups considered more vulnerable, including adolescents, pregnant women, and foetuses, whereas most national HBM programs focus on adults where sampling is more accessible. The recruitment and sample collection covers an extensive period of 17 years for assessing time trends, which includes important changes with implemented regulations and initiatives aiming to reduce human exposure. Furthermore, we have provided new toxicokinetic data based on one of the few human experimental exposure studies, that can improve future interpretation of biomonitoring data from other populations for glyphosate. The final study assessed TTE of PFAS in a highly exposed MotherChild cohort, where we were able to compare if maternal exposure concentrations impact the transfer, which has not been studied previously. Additionally, findings based on higher population concentrations are more reliable and less impacted by difficulties in analytical measurements compared to concentrations close to the LOD or LOQ.

The sample integrity and compound stability of long-term stored urine and serum samples for specific compounds were assessed by comparing the analytical results after up to 6 years of storage. Since analyses are costly and time-consuming, such comparisons are not often performed even though retrospective studies including samples stored in biobanks are commonly used for human biomonitoring purposes. Our comparison indicates that both the stability of biomarkers and the integrity of such samples are maintained.

However, the thesis also has some limitations. One shortcoming is related to sampling procedures, where spot urine samples were used and analysed for pesticides with short biological half-lives, although intra-individual variability might have resulted in non-differential misclassification of the exposure. Furthermore, the total sample size differs between urine (Study I) and serum

samples (Study III) from adolescents, especially for the earlier years 2000 and 2009 (Figure 2). The explanation is that the total sample volume collected was not sufficient for analyses including all pesticide biomarkers.

In study IV, the sampling procedure for maternal serum during pregnancy deviated from the original protocol assuming the samples would be collected earlier and would represent a more valid estimate of maternal exposure, less affected by pregnancy-related physiological processes. Furthermore, the sampling procedure of umbilical cord serum was not standardized, which limits the ability to explain the wide range of cord concentrations observed for certain compounds. Harmonized sampling protocols for the assessment of different exposure scenarios need to be further developed within the scope of joint human biomonitoring efforts, where sampling procedures of groups recognized as more vulnerable (such as foetuses/infants) should be prioritized.

The external validity of human biomonitoring data, i.e., the generalizability, of estimates obtained from cohorts to the general population may represent a challenge if participation is selective and the cohort does not represent the target population. However, background exposed individuals of the general population, such as the participants in studies I and III, are not aware of their internal exposure levels and participation is rarely conditioned on exposure (although participants may have been more environmentally aware). In study IV, all pregnant women in the exposed area were offered to participate and were thereby eligible for inclusion. The serum levels in the cohort confirmed a wide range of exposures. The estimated TTE was calculated based on matched maternal-cord serum samples as a relative measure, and selection bias is therefore not a concern in study IV.

Regarding the cohorts of adolescents, a major change in population composition was introduced after year 2009 when both men and women were included. Men and women may differ with respect to both lifestyle patterns and elimination half-lives impacting their internal exposure concentrations. However, as we hypothesised that differences in exposure patterns between men and women may impact the estimated trends, we performed a sensitivity analysis excluding women in both studies I and III. We found no effects on the model estimates.

The oral exposure experiment was limited to only three volunteers and high inter-individual differences were observed. The sample size was a trade-off between the ethical concern related to the intentional administration of a potentially toxic chemical and a desire to reduce inter-individual variation in kinetics. Considering that human toxicokinetic data for glyphosate is very scarce, the results are still valuable for our understanding and interpretation of human biomonitoring data, despite being limited to three participants.

Future research perspectives

Biomonitoring programs have made more information available on human exposure to environmental contaminants and it is important that this work remains prioritized and supported by both national and international politics. The interpretation of this data lags with a need for derived guidance values relevant to general populations and vulnerable subgroups, including transgenerational effects such as prenatal exposure. Such values need to be further supported by toxicokinetic data for substances representing major compound groups. Small-scale human volunteer studies can provide important toxicokinetic data that aids both method development and interpretation of data, but such studies are rare and usually not ethically accepted. Still, we need to know more about the toxicokinetics of widely used compounds, such as glyphosate, to determine if it is absorbed and accumulates in the body to distinguish the biologically relevant exposures. The demand for increased knowledge of both compound-specific details as well as overall interpretation and communication of data within the multidisciplinary field of HBM requires extended collaborations, where both specialized and broad competence is needed.

The substances prioritized in established human biomonitoring programs are based on current knowledge of health effects, population exposure, and available and verified analytical methods. Therefore, more experimental, and non-targeted screening studies might be needed to include less studied compounds where toxicity is still unclear. As the monitoring of occupational exposure is anchored in legislation, it should be an incentive to develop analytical methods for assessing work-related exposure and those methods could be further used for the biomonitoring of population exposure.

Finally, the majority of existing human biomonitoring programs are mainly developed and focused on western countries in Europe or North America, where legislative frameworks for chemical safety are already in place. Production and use of compounds that have been phased out or restricted in these areas are still detected in imported items or consumer products, and the lack of international regulations leads to a complex pollution scenario (Fama et al. 2022). It is therefore highly relevant to focus on the development of human biomonitoring programs in other parts of the world to promote equal chemical safety regulations.

Conclusions

The overall conclusions are that there is a low but widespread population exposure to pesticides and PFAS in Sweden, although trends in exposure generally coincide with implemented restrictions. The results also advance current knowledge of key components of human biomonitoring, including compound-specific characteristics of elimination kinetics, transplacental transfer at different exposure levels, and stability and sample integrity after storage, which are key components of human biomonitoring. Taken together, the results of the thesis facilitate future interpretation of exposure data.

- Human biomonitoring through repeated cross-sectional of urinary pesticide biomarkers indicated widespread exposure in adolescents in southern Sweden. The restricted insecticide chlorpyrifos (TCPy) was detected in >99% of the samples whereas glyphosate, the most used herbicide globally, was detected in 20% in year 2017.
- Decreasing trends were observed for biomarkers of fungicides (tebuconazole and ETU) and the growth regulator chlormequat between years 2000 and 2017, likely explained by replacements with new substitutes.
- Widespread exposure to low levels of PFHxS, PFOS, PFOA, PFNA, and PFDA was detected in the serum of Swedish adolescents.
- Decreasing trends were observed for PFHxS, PFOS, and PFOA throughout years 2000 – 2017, which is consistent with the timing of international initiatives to phase out these compounds.
- PFAS remained stable in serum samples when compared with results from a repeated analysis after up to 6 years of storage.
- Elimination of glyphosate in urine followed a two-phase excretion and the oral dose recovery was 1-6%, considerably lower than previous estimations at 20% from animal studies. The metabolite AMPA was found in very low concentrations with a moderate correlation with glyphosate, indicating separate exposures.
- Estimated TTE of PFAS in mothers with a wide range of exposures suggests marginally lower transfer efficiencies in highly exposed individuals,

implying higher absolute fetal exposure and a need for further research on child health and development in areas with a point source of exposure.

Acknowledgements

Tack till min huvudhandledare **Christian Lindh** för allt du lärt mig, för att du gett mig både frihet och ansvar, låtit (tvingat) mig att vara självständig som doktorand och för att jag fått göra sådant jag tycker är kul! Tack också för att du är så knäpp, hade aldrig föreställt mig att jag skulle få skratta lika mycket som jag forskar under min doktorandtid.

Tack till min handledare **Christel Nielsen**, att din roll i mitt projekt har vuxit kontinuerligt under dessa år är jag otroligt tacksam för! Med en kompetens lika vass som din humor har du gjort att jag alltid sett fram emot våra möten, av flera anledningar. Jag hoppas att tramsklubben kommer leva vidare (även om denna handledarkonstellation kanske inte gör det nu när jag äntligen är i mål!)

Emelie Rietz-Liljedahl, en bättre doktorandkollega får man leta efter. Du har varit en trygghet som stöttat och hjälpt mig med allt sedan dag ett, det är en ära att få dela kontor med dig. Tack för alla skratt och för att du alltid funnits där!

Annette Kraiss and **Daniela Pineda**, thank you for every lunch, laugh, and after-work! You're the best colleagues and friends one can have, you've always helped me with everything, including chemical analyses, moral support, sharing your secret chocolate storage, and watering my plants.

Maggan Littorin, en favoritperson utan tvekan. Jag slutar aldrig imponeras av din ständiga nyfikenhet, envishet och att du aldrig slutar ställa frågor! Är väldigt glad att jag har lärt känna dig och att vi kan fortsätta prata om både rosor och politik.

Stort tack till **Magg, Åsa, Agneta, Hamideh, Marie** och **Anna**! Ni har inte bara analyserat alla prover i min avhandling, ni har också varit så fina, omtänksamma, roliga och stöttande kollegor alltid.

Tack till de otroliga sköterskorna **Else, Eva** och **Ulla** som inte missat ett enda nålstick under provinsamlingarna. En ynnest att få ha med sådana proffs, som dessutom gjort provinsamlingarna till något roligt jag sett fram emot trots att jag egentligen varit stressad!

Vill tacka både **Estelle Larsson** och **Jonatan Axelsson** för ert bidrag till denna avhandling och att ni stöttade mig som handledare när jag var en ny och förvirrad doktorand!

Lars Rylander, tack för ditt bidrag som medförfattare men främst för att jag fick anordna en konferens tillsammans med dig, en höjdpunkt jag sent kommer glömma!

Tack **Anders Glynn** för ett fint samarbete med artiklar, att du bjöd in mig att hälsa på er på SLU i Uppsala och för att jag fått höra lite härligt hälsingemål under tiden!

Thank you **Annelise** for your input and contribution to my thesis! I'm very happy and grateful that such a competent and nice colleague started working here during the final year of my PhD. Cheers!

Tack till alla doktorandkollegor och medarbetare på AMM (nuvarande och tidigare) som gjort detta till en så spännande och rolig arbetsplats!

Tack **Paulina Nordling** som förutom att vara min bästa vän även har designat den fina framsidan till denna avhandling (en bok döms trots allt efter omslaget).

Tack till **Natalie, Filipa** och alla fantastiska vänner som funnits där, hejat på och peppat mig under alla år!

Tack till **Daniel Hansson** och alla vänner i **Malmö Akademiska Kör och Orkester** som varit ett viktigt och fint sammanhang som gett mig energi och glädje genom detta!

Och tack alla vänner och medlemmar i mitt bästa tramsiga band **Discokollektivet**, vad vore livet utan er och paljetter?

Tack till **mamma** och **pappa** för att ni alltid inspirerat och stöttat mig och lagt grunden för hela mitt miljöintresse och engagemang! Och tack till **min system** för att du är den smartaste person jag vet som fortsätter lära mig nya saker hela tiden.

Vill också tacka mina svärföräldrar **Kim** och **Set**, er hjälp med barnpassning har varit ovärderlig och en förutsättning för att jag kunnat slutföra detta.

Slutligen vill jag tacka min fantastiska lilla familj. **Rasmus**, ett bättre stöd hade jag inte kunnat få! Tack för ditt tålamod och lugn som gjort att jag tagit mig igenom allt i livet den här tiden, jag hade aldrig klarat det utan dig. Lilla **Natanael**, sömnbristen du orsakat har varit påtaglig men längtan hem till dig har varit min största drivkraft i detta!

References

- Abreu-Villaça Y and Levin ED. 2017. Developmental neurotoxicity of succeeding generations of insecticides. *Environ Int*; 99: 55 – 77.
- Anadón A, Martínez-Larranga MR, Martínez MA, Castellano VJ, Martínez M, Martín MT, Nozal MJ, Bernal JL. 2009. Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. *Toxicol Lett*; 190(1): 91 – 95.
- Angerer J, Bird MG, Burke TA, Doerrner NG, Needham L, Robinson SH, Sheldon L, Zenick H. 2006. Strategic Biomonitoring Initiatives: Moving the Science Forward. *Toxicological Sciences*; 93(1): 3 – 10.
- Angerer J, Ewers U, Wilhelm M. 2007. Human biomonitoring: State of the art. *Int J Hyg Environ Health*; 210(3): 201 – 228.
- Apel P, Rousselle C, Lange R, Sissoko F, Kolossa-Gehring M, Ougier E. 2020. Human biomonitoring initiative (HBM4EU) – Strategy to derive human biomonitoring guidance values (HBM-GVs) for health risk assessment. *Int J Hyg Environ Health*; 230: 11362
- Bahadori T, Phillips RD, Money CD, Quackenboss JJ, Clewell HJ, Bus JS, Robinson SH, Humphris CJ, Parekh AA, Osborn K, Kauffman RB. 2007. Making sense of human biomonitoring data: findings and recommendations of a workshop. *J Expo Sci Environ Epidemiol*; 17(4): 308 – 313.
- Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. 2005. Urinary creatinine concentrations in the US population: Implications for urinary biologic monitoring measurements. *Environ Health Persp* 113: 192 – 200.
- Becker K, Seiwert M, Casteleyn L, Joas R, Joas A, Biot P, Aerts D, Castaño A, Esteban M, Angerer J, Koch HM, Schoeters G, Den Hond E, Sepai O, Exley K, Knudsen LE, Horvat M, Bloemen L, Kolossa-Gehring M. 2014. A systematic approach for designing a HBM Pilot Study for Europe. *Int J Hyg Env Health*; 217(2-3): 312 – 322.
- Bernhardt ES, Rosi EJ, Gessner MO. 2017. Synthetic chemicals as agents of global change. *Frontiers in Ecology and The Environment*; 15(2): 84 – 90.
- Bevan R, Brown T, Matthies F, Sams C, Jones K, Hanlon J, La Vedrine M. 2017. Human biomonitoring data collection from occupational exposure to pesticides. EFSA Supporting Publication 2017: EN-1185.

- Bil W, Govarts E, Zeilmaker MJ, Woutersen M, Bessems J, Ma Y, Thomsen C, Haug LS, Lignell S, Gyllenhammar I, Palkovicova Murinova L, Fabelova L, Snor Tratnik J, Kosjek T, Cabriel C, Sarigiannis D, Pedraza-Díaz S, Esteban-López M, Castano A, Rambaud L, Riou M, Franken C, Colles A, Vogel N, Kolossa-Gehring M, Halldorsson TI, Uhl M, Schoeters G, Santonen T, Vinggaard AM. 2022. Approaches to mixture risk assessment of PFASs in the European population based on human hazard and biomonitoring data. *Int J Hyg Environ Health*; 247: 114071.
- Bradman A, Quiros-Alcala L, Castorina R, Aguilar Schall R, Camacho J, Holland NT, Barr DB, Eskenazi B. 2015. Effect of organic diet intervention on pesticide exposures in young children living in low-income urban and agricultural communities. *Environ Health Perspect*; 123(10): 1086 – 1093.
- Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, Jensen AA, Kannan K, Mabury SA, van Leeuwen SP. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr Environ Assess Manag*; 7(4): 513 – 341.
- Buekers J, Remy S, Bessems J, Govarts E, Rambaud L, Riou M, Halldorsson TI, Ólafsdóttir K, Probst-Hensch N, Ammann P, Weber T, Kolossa-Gehring M, Esteban-López M, castaño A, Raun Andersen H, Schoeters G. 2022. Glyphosate and AMPA in Human Urine of HBM4EU-Aligned Studies: Part B Adults. *Toxics*; 10(10): 552. DOI: <https://doi.org/10.3390/toxics10100552>
- Chambers WS, Hopkins JG, Richards SM. 2021. A Review of Per- and Polyfluorinated Alkyl Substance Impairment of Reproduction. *Front Toxicol*; 3: 732436.
- Carson R. 1962. *Silent Spring*. Boston, MA: Houghton Mifflin.
- CDC. 2020. Available online: https://www.cdc.gov/nchs/nhanes/about_nhanes.htm (accessed on 31 October 2022)
- Connolly A, Coggins MA, Koch HM. 2020. Human Biomonitoring of Glyphosate Exposures: State-of-the-Art and Future Research Challenges. *Toxics*; 8(3): 32824707.
- Cousins IT, DeWitt JC, Glüge J, Goldenman G, Herzke D, Lohmann R, Ng CA, Scheringer M, Wang Z. 2020. The high persistence of PFAS is sufficient for their management as a chemical class. *Environ Sci Process Impacts*; 22(12): 2307 – 2312.
- Dalsager L, Christensen N, H alekoh U, Timmermann CAG, Nielsen F, Kyhl HB, Husby S, Grandjean P, Jensen TK, Andersen HR. 2021. Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Cohort. *Environ Int*; 149: 106395.
- EEA-JRC, Environment and human health, Joint EEA-JRC Report. European Environment Agency Report No 5/2013, Copenhagen, Denmark, 2013
- European Environment Agency. 2018. HBM4EU priority substances and the prioritisation strategy. Available online: <https://www.eea.europa.eu/themes/human/human-biomonitoring/prioritisation-and-substances>, Accessed 2022-11-04
- Fama F, Feltracco M, Moro G, Barbaro E, Bassanello M, Gambaro A, Zanardi C. 2022. Pesticides monitoring in biological fluids: Mapping the gaps in analytical strategies. *Talanta*; 253: 123969.

- Ganzleben C, Antignac J-P, Barouki R, Castaño A, Fiddicke U, Klánová J, Lebret E, Olea N, Sarigiannis D, Schoeters GR, Sepai O, Tolonen H, Kolossa-Gehring M. 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. *Int J Hyg Environ Health*; 220: 94 – 97.
- Gyllenhammar I, Glynn A, Jonsson BA, Lindh CH, Darnerud PO, Svensson K, Lignell S. 2017. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? *Environ Res*; 153: 48 – 54.
- Göen T, Schaller KH, Drexler H. 2012. External quality assessment of human biomonitoring in the range of environmental exposure levels. *Int J Hyg Environ Health*; 215(2): 229 – 232.
- Hays SM, Aylward LL, Blount BC. 2015. Variation in Urinary Flow Rates According to Demographic Characteristics and Body Mass Index in NHANES: Potential Confounding of Associations between Health Outcomes and Urinary Biomarker Concentrations. *Environ Health Persp* 123(4): 293 – 300.
- Hoet P and Haufroid V. 1997. Biological monitoring: state of the art. *Occup Environ Med*; 54(6): 361 – 366.
- Howe, G.M. 1980. *The environment, Its Influences and Hazards to Health in Environmental Medicine* (Second Edition). Editor: Howe G.M. & Loraine J.A. Butterworth-Heinemann.
- International Ethical Guidelines for Health-related research Involving Humans, Fourth Edition. Geneva. Council for International Organizations of Medical Sciences (CIOMS); 2016.
- Joas R, Casteleyn L, Biot P, Kolossa-Gehring M, Castano A, Angerer J, Schoeters G, Sepai O, Knudsen L, Joas A, Horvat M, Bloemen L. 2012. Harmonised human biomonitoring in Europe: activities towards an EU HBM framework. *Int J Hyg Environ Health*; 215(2):172 – 175.
- Jeddi MZ, Hopf NB, Louro H, Viegas S, Galea KS, Pasanen-Kase R, Santonen T, Mustieles V, Fernandez MF, Verhagen H, Bopp SK, Antignac JP, David A, Mol H, Barouki R, Audouze K, Duca R-C, Frantke P, Scheepers P, Ghosh M, Van Nieuwenhuyse A, Vicente JL, Trier X, Rambaud L, Fillol C, Denys S, Conrad A, Kolossa-Gehring M, Paini A, Arnot J, Schulze F, Jones K, Sepai O, Ali I, Brennan L, Benfenati E, Cubadda F, Mantovani A, Bartonova A, Connolly A, Slobodnik J, Bruinen de Bruin Y, van Klaveren J, Palmén N, Dirven H, Husøy T, Thomsen C, Virgolino A, Rössli M, Gant T, von Goetz N, Bessems J. 2022. Developing human biomonitoring as a 21st century toolbox within the European exposure science strategy 2020 – 2030. *Environ Int* 168: 107476.
- Jansson A and Fohgelberg P. 2018. Kontroll av bekämpningsmedelsrester i livsmedel 2016. Sweden: National Food Agency; Rapportserie nr 8 2018. Available online: <https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2018/2018-nr-8-kontroll-av-bekampningsmedelsrester-i-livsmedel-2016.pdf>. Accessed 5th of December 2022.
- Jiang Q, Gao H, Zhang L. 2015. Metabolic Effects PFAS. In: DeWitt, J (eds) *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Molecular and Integrative Toxicology*. Humana Press, Cham.

- Johnson CL, Paulose-Ram R, Ogden CL, Carroll MD, Kruszon-Moran D, Dohrmann SM, Curtin LR. 2013. National health and nutrition examination survey: analytic guidelines, 1999 – 2010. *Vital Health Stat 2*. 161:1 – 24.
- Kehoe RA, Thamann F, Cholack J. 1933. Lead absorption and excretion in certain trades. *J Ind Hyg* 15: 306 – 319.
- Kissa, E. 2001. *Fluorinated Surfactants and Repellents* Vol. 97, 616 pp.
- Knudsen, L. 2021. The role of human biomonitoring in assessing and managing chemical risks in the Nordic countries: HBM4EU – The European Human Biomonitoring; and its continuation in PARC. Nordic Counsel of Ministers, June 2021, 64 pp.
- Kuipers Y and Mascolo M. 2017. Study for the strategy for a non-toxic environment of the 7th EAP, Sub-study c: the protection of vulnerable groups, August 2017/11.
- Louro H, Heinälä M, Bessems J, Buekers J, Vermeire T, Woutersen M, van engelen J, Borges T, Rousselle C, Ougier E, Alvito P, Martins C, Assunção R, Silva MJ, Pronk A, Schaddelee-Scholten B, Gonzales MDC, de Alba M, Castaño A, Viegas S, Humar-Juric T, Kononenko L, Lampen a, Vinggaard AM, Schoeters G, Kolossa-Gehring M, Santonen T. 2019. *Int J Hyg Environ Health*; 222(5): 727 – 737.
- Manno M, Viau C, Cocker J, Colosio C, Lowry L, Mutti A, Nordberg M, Wang S. 2010. Biomonitoring for occupational health risk assessment (BOHRA). *Toxicol Lett*; 192(1): 3 – 16.
- Mårtensson AL. 2019. Tillväxtreglerare i spannmål 1992 – 2017—en trendanalys. Uppsala: National Food Agency, Sweden—Livsmedelsverkets rapportserie; nr 03 2019.
- Needham LL and Sexton K. 2000. Assessing children’s exposure to hazardous environmental chemicals: An overview of selected research challenges and complexities. *J Expo Anal Environ Epidemiol* 10:611 – 629.
- Nguyen VK, Colacino JA, Arnot JA, Kvasnicka J, Jolliet O. 2019. Characterization of age-based trends to identify chemical biomarkers of higher levels in children. *Environ Int*; 122: 117 – 129.
- Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I. 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication:159 pp.
- Nübler S, Esteban López M, Castaño A, Mol HGJ, Haji-Abbas-Zarrabi K, Schäfer M, Müller J, Hajslova J, Dvorakova D, Antignac J-P, Koch HM, Småstuen Haug L, Vorkamp K, Göen T. 2022. Interlaboratory Comparison Investigations (ICIs) and External Quality Assurance Schemes (EQUASs) for human biomonitoring of perfluoroalkyl substances (PFASs) in serum as part of the quality assurance programme under HBM4EU. *Sci Total Environ*; 847: 157481. DOI: 10.1016/j.scitotenv.2022.157481
- Oates L, Cohen M, Braun L, Schembri A, Taskova R. 2014. Reduction in urinary phosphate pesticide metabolites in adults after a week-long organic diet. *Environmental research* 132:105 – 111.
- Organisation for Economic Cooperation & Development / United Nations Environment Programme. 1999. *Phasing Lead Out of Gasoline: an examination of policy approaches in different countries*. Paris: OECD; 1999.

- Ougier E, Ganzleben C, Lecoq P, Bessems J, David M, Schoeters G, Lange R, Meslin M, Uhl M, Kolossa-Gehring M, Rousselle C, Vicente JL. 2021. Chemical prioritization strategy in the European Human Biomonitoring Initiative (HBM4EU) – Development and results. *Int J Hyg Environ Health*; 236: 113778.
- Pearson MA, Lu C, Schmotzer BJ, Waller LA, Riederer AM. 2009. Evaluation of physiological measures for correcting variation in urinary output: Implications for assessing environmental chemical exposure in children. *J Expo Sci Environ Epidemiol*; 19: 336 – 342.
- Pollock T, Karthikeyan S, Walker M, Werry K, St-Amand A. 2021. Trends in environmental chemical concentrations in the Canadian population: Biomonitoring data from the Canadian Health measures Survey 2007 – 2017. *Environ Int* 2021; 155: 106678. DOI: 10.1016/j.envint.2021.106678
- Prüss-Ustun A, Vickers C, Haefliger P, Bertollini R. 2011. Knowns and unknowns on burden of disease due to chemicals: a systematic review. *Environ Int*; 10(1): 9.
- Richterová D, Govarts E, Fábelová L, Rausová K, Rodriguez Martin L, Gilles L, Remy S, Colles A, Rambaud L, Roiu M, Gabriel C, Sarigiannis D, Pedraza-Diaz S, Ramos JJ, Kosjek T, Snoj Tratnik J, Lignell S, Gyllenhammar I, Thomsen C, Haug LS, Kolossa-Gehring M, Vogel N, Franken C, Vanlarebeke N, Bruckers L, Stewart L, Sepai O, Schoeters G, Uhl M, Castaño A, Esteban López, Göen T, L'Palkovicová Murínová. 2022. PFAS levels and determinants of variability in exposure in European teenagers – Results from the HBM4EU aligned studies (2014 – 2021). *Int J Hyg Environ Health*; 247: 114057
- Saillenfait AM, Ndiaye D, Sabaté JP. 2015. Pyrethroids: exposure and health effects—an update. *Int J Hyg Environ Health*; 218(3): 281 – 292.
- Saillenfait AM, Ndiaye D, Sabaté JP. 2016. The estrogenic and androgenic potential of pyrethroids in vitro. Review. *Toxicol In Vitro*; 34: 321 – 332.
- Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, Hogstrand C, Hoogenboom IR, Leblanc J-C, Nebbia CS, Nielsen E, Ntzanti E, Petersen A, Sand S, Vleminckx C, Wallace H, Varrgård L, Ceccatelli S, Cravedi J-P, Halldorsson TI, Haug LS, Johansson N, Knutsen HK, Rose M, Roudot A-C, van Loveren H, Vollmer G, Mackay K, Riolo F, Schwerdtle T. 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA J*. 18. 6223 – 6391.
- Schulz C, Conrad A, Becker K, Kolossa-Gehring M, Seiwert M, Seifert B. 2007. Twenty years of the German Environmental Survey (GerES): Human biomonitoring – Temporal and spatial (West Germany/East Germany) differences in population exposure. *Int J Hyg Environ Health*, 2010: 271 – 297.
- Schumann M, Lilienthal H, Hölzer J. Human biomonitoring (HBM)-II values for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) – Description, derivation and discussion. *Regul Toxicol Pharmacol*; 121: 104868
- Sieke C. 2018. Probabilistic cumulative dietary risk assessment of pesticide residues in foods for the German population based on food monitoring data from 2009 to 2014. *Food Chem Toxicol*; 121: 296 – 403.

- Spratlen MJ, Perera FP, Lederman SA, Rauh VA, Robinson M, Kannan K, Trasande L, Herbstman J. 2020. The association between prenatal exposure to perfluoroalkyl substances and childhood neurodevelopment. *Environ Pollut*; 264 (Pt B): 114444.
- Tarazona JV, González-Caballero MDC, Alba-Gonzalez M, Pedraza-Diaz S, Cañas A, Dominguez-Moruco N, Esteban-López M, Cattaneo I, Katsonouri A, Makris KC, Halldorsson TI, Olafsdottir K, Zock J-P, Dias J, De Decker A, Morrens B, Berman T, Barnett-Itzhaki Z, Lindh C, Gilles L, Govarts E, Schoeters G, Weber T, Kolossa-Gehring M, Santonen T, Castaño A. Improving the Risk Assessment of Pesticides through the Integration of Human Biomonitoring and Food monitoring Data: A case study for chlorpyrifos. *Toxics*; 10(6): 313. <https://doi.org/10.3390/toxics10060313>
- Tranfo, G. 2020. The growing importance of the Human Biomonitoring of Exposure. *Int J Environ Res Public Health* 17(11):3934. <https://doi.org/10.3390/ijerph17113934>
- United Nations Environment Programme. 2013. Global Chemicals Outlook – Towards Sound Management of Chemicals. <https://wedocs.unep.org/20.500.11822/8455>
- Vorkamp K, Castaño A, Antignac J-P, Boada LD, Cequier E, Covaci A, López, ME, Haug LS, Kasper-Sonnenberg M, Koch HM, Luzardo OP, Osite A, Rambaud L, Pinorini M-T, Sabbioni G, Thomsen C. 2021. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. *Environ Int*; 146: 106082.
- Wittsiepe J, Schrey P, Ewers U, Selenka F, Wilhelm M. 2000. Decrease of PCDD/F levels in human blood from Germany over the past ten years (1989 – 1998). *Chemosphere*; 40:1103 – 1109.
- Xu Y, Nielsen C, Li Y, Hammarstrand S, Andersson EM, Li H, Olsson DS, Engström K, Pineda D, Lindh CH, Fletcher T, Jakobsson K. 2021. Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby, Sweden. *Environ Int*; 147: 106333.
- Zidek A, Macey K, MacKinnon L, Patel M, Poddalgoda D, Zhang Y. A review of human biomonitoring data used in regulatory risk assessment under Canada's Chemicals Management Program. *Int J Hyg Environ*; 220(2): 167 – 178.
- Zielhuis RL. 1984. Recent and Potential Advances Applicable to the Protection of Workers' Health – Biological Monitoring II. In: Berlin A, Yodaiken RE, Henman BA (eds) *Assessment of Toxic Agents at the Workplace*. Springer, Dordrecht.
- Zoller O, Rhyn P, Zarn JA, Dudler V. 2020. Urine glyphosate level as a quantitative biomarker of oral exposure. *Int J Hyg Environ Health*; 228: 113526
- Yant Wp, Schrenk HH, Sayers RR, Howarth AA, Reinhart WA. 1936. Urine sulfate determination as a measure of benzene exposure. *J Ind Hyg Toxicol* 18: 69.
- Yusa V, Millet M, Coscolla C, Roca M. 2015. Analytical methods for human biomonitoring of pesticides. A review. *Anal Chim Acta* 891: 15 – 31.