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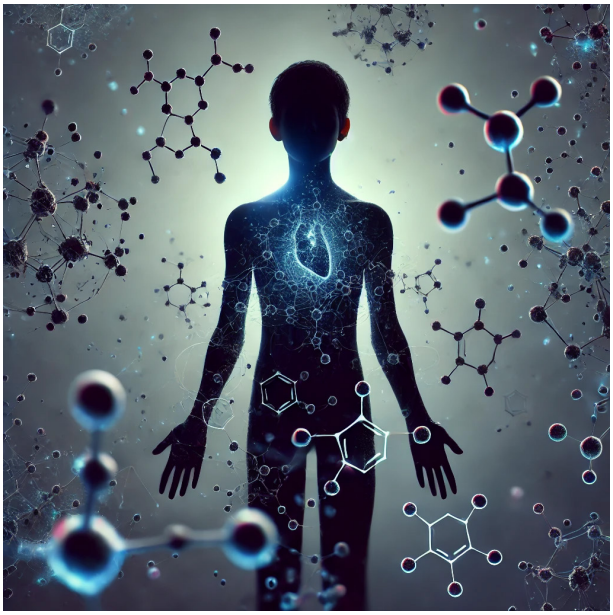
Understanding chemical mixtures in Swedish adolescents

An epidemiological investigation into exposure, socio-demographic determinants and immune-health effects

Sebastian Pineda



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Cover: Adolescent surrounded by chemicals, created using DALL·E 3

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Abstract

The world's human population is ubiquitously exposed to a complex mixture of chemicals. This thesis aims to analyse adolescent exposure in Sweden to a complex chemical mixture, including essential and non-essential elements, persistent organic pollutants (POPs), phthalates/phthalate alternatives, phosphorous flame retardants, and phenolic compounds. The research also investigated potential associations between this chemical mixture exposure and adolescent vaccination antibody titres of diphtheria, tetanus, pertussis, measles, and rubella. The study population (N=1082), Riksmaten Adolescents 2016-17 (RMA), were found to have similar chemical exposure levels as adolescents from Germany, Belgium, and the USA. Large population exceedances of biomonitoring guidance values were identified for lead, aluminium and per- and poly-fluoroalkyl substances. A conservative mixture risk assessment indicated that the effects of some of the investigated chemicals on the central nervous system may be a health concern. The level of mixture exposure varied based on factors such as age, gender, country of birth, parental education level, and longitude/latitude of the participant's home address. Birth country was identified as the most influential determinant. Mixture exposure to elements and POPs was linked to decreased diphtheria, tetanus and pertussis antibody titres whilst the opposite was observed for measles and rubella antibody titres. However, the observed associations were weak and uncertain. This study revealed disparities in mixture exposure among Swedish adolescents born in different countries as well as gender and regional exposure differences. Addressing these disparities in mixture exposure is essential to achieve the United Nations sustainability goals of equality within and between countries and gender equality.

Keywords: biomonitoring, chemical mixture, vaccines, adolescents, risk assessment

En epidemiologisk undersökning av svenska ungdomars exponering för kemikalieblandningar - sociodemografiska bestämningsfaktorer och effekter på immunhälsa

Abstrakt

Världens befolkning exponeras för en komplex blandning av kemikalier. Denna avhandling syftar till att analysera ungdomars exponering i Sverige för essentiella och icke-essentiella grundämnen, persistenta organiska föroreningar (POP), ftalater/ftalatalternativ, fosfor flamskyddsmedel och fenolföreningar. Forskningen undersökte också potentiella samband mellan exponering för denna kemiska blandning och antikroppstitrar mot difteri, stelkramp, kikhosta, mässling och röda hund. Studiepopulationen (N=1082), Riksmaten Ungdom 2016-17 (RMU), visade sig ha liknande kemikalieexponeringsnivåer som ungdomar från Tyskland, Belgien och USA. En andel av RMA-deltagarna hade exponeringsnivåer i nivå eller över hälsobaserade riktvärden för biomonitorering gällande bly, aluminium och per- och polyfluorerade alkylsubstanser (PFAS). En konservativ riskbedömning av blandningarna visade att effekterna av några av de undersökta kemikalierna på det centrala nervsystemet behöver undersökas vidare. Nivån på blandningsexponeringen varierade beroende på faktorer som ålder, kön, födelseland, föräldrarnas utbildningsnivå och longitud/latitud för deltagarens hemadress. Födelselandet identifierades som den mest inflytelserika faktorn. Blandningsexponering för grundämnen och POPs var kopplad till minskade titrar av difteri-, stelkramp- och pertussisantikroppar medan motsatsen observerades för antikroppstitrar mot mässling och röda hund. De observerade sambanden var dock svaga och osäkra. Denna studie avslöjade skillnader i exponering bland svenska ungdomar födda i olika länder samt köns- och regionala exponeringsskillnader. Att åtgärda dessa skillnader i exponering är väsentligt för att uppnå FN:s hållbarhetsmål om jämställdhet inom och mellan länder och mellan könen.

Nyckelord: biomonitorering, kemiska blandningar, exponering, ungdomar

Dedication

To los Osos

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Pineda, S**., Lignell, S., Gyllenhammar, I., Lampa, E., Benskin, J.P., Lundh, T., Lindh, C., Kiviranta, H. & Glynn, A. (2023). Exposure of Swedish adolescents to elements, persistent organic pollutants (POPs), and rapidly excreted substances – The Riksmaten adolescents 2016-17 national survey. *International Journal of Hygiene and Environmental Health*, 251 (114196)

II. **Pineda, S**., Lignell, S., Gyllenhammar, I., Lampa, E., Benskin, J.P., Lundh, T., Lindh, C., Kiviranta, H. & Glynn, A. (2024). Socio-demographic inequalities influence differences in the chemical exposome among Swedish adolescents. *Environment International*, 186 (108618)

III. **Pineda, S**., Lignell, S., Gyllenhammar, I., Lampa, E., Benskin, J.P., Lundh, T., Kiviranta, H. & Glynn, A. Associations between chemical mixtures and vaccination antibody titres in Swedish adolescents (manuscript)

The contribution of Sebastian Pineda (**SP**) to the papers included in this thesis was as follows:

I. **SP** was involved in the planning and conceptualisation of the paper. All data wrangling and statistical analysis was done by **SP**. **SP** wrote the first and final draft, with input from fellow authors.

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Abbreviations

AAM	Division of Occupational and Environmental Medicine
Al	Aluminium
BBzP	Butyl-benzyl-phthalate
BKMR	Bayesian kernel machine regression
BFR	Brominated flame retardants
BHA	Butylated hydroxyanisole
BPA	Bisphenol A
BP-3	Benzophenone-3
Co	Cobalt
Cr	Chromium
Cd	Cadmium
CNS	Central nervous system
DBP	Di-butylphthalate
DDT	Dichloro-diphenyl-trichloroethane
DDE	Dichloro-diphenyl-dichloroethylene
DEHP	Bis(2-ethylhexyl)phthalate
DINP	Di-isononylphthalate
DTP	Diphtheria, Tetanus, Pertussis
HBGV	Health-based guidance values
HBM-GV	Human biomonitoring guidance values
HCB	Hexachlorobenzene
Hg	Mercury
HI	Hazard index
HR	Hazard ratios
MCMC	Markov chain Monte Carlo
Mn	Manganese

MR	Measles, Rubella
Ni	Nickle
OCP	Organochlorine pesticides
ORM	Ordinal regression models
PAH	Polycyclic aromatic compounds
Pb	Lead
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PFAA	Perfluorinated alkyl acids
POP	Persistent organic pollutant
PFR	Phosphorus flame retardant
RMA	Riksmaten Adolescents 2016-17
Se	Selenium
TWI	Tolerable weekly intake
QGC	Quantile g-computation
3-PBA	3-Phenoxybenzoic acid
4,4-BPF	Bisphenol F

1. Introduction

The use of chemicals is ubiquitous within our modern-day society. From the clothes we wear to the food we eat or its packaging to even the natural environment, toxic chemical exposure is unavoidable. Yet chemical production is vital for the continued industrialisation of the world, and sales are projected to double from 2017 to 2030 (EEA 2024). Various technological advancements leading us into our modern-day life would have been impossible without chemicals. But with over 300,000 chemicals currently registered across 22 chemical inventories (Wang et al. 2020), many potentially toxic to humans, the risk of toxic mixture effects on the general population will continue to grow.

1.1 Chemical mixtures

The study of chemical mixtures is a critical field in toxicology that addresses the complexities and interactions of multiple chemical substances when combined. Unlike individual chemicals, mixtures exhibit unique properties and behaviours that cannot be predicted solely by understanding their isolated components (Martin et al. 2021). This area of research is vital for several reasons: it has profound implications for environmental, food and drinking water safety since potentially toxic substances often exist as complex mixtures rather than single compounds (Livsmedelsverket 2017; Schaap et al. 2023; Rosenblum et al. 2024), and it is essential in the pharmaceutical industry for the development of effective drug combinations (Cheng et al. 2019). Understanding the toxicity of chemical mixtures is fundamental to ensuring the safety, efficacy, and sustainability of chemical production and use.

Within this thesis, we will examine adolescent exposure to several different chemical groups, many of which are typically found in food common to Sweden (Tables 1 & S.2, **Paper I**) (Livsmedelsverket 2017). The chemical groups include substances which have relatively long half-lives in the body (months/years), comprising of essential and non-essential elements as well as persistent organic pollutants (POPs): organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), per- and poly-fluoroalkyl substances (PFAS), and polybrominated diphenyls (PBDE). Moreover, our study also includes substances with very short half-lives in the body (hours/days) that are suspected to be ubiquitously present in the adolescent environment (Dhanirama et al. 2012; Poma et al. 2017; Svensson et al. 2024), including phthalates/phthalate alternatives, bisphenols, phosphorous flame retardants (PFR), pesticides, polycyclic aromatic compounds (PAH), triclosan (TCS), butylated hydroxyanisole (BHA) and benzophenone-3 (BP-3) (Table 1 & S.2, **Paper I**). A full list of substances included in this thesis, with full names, abbreviations, CAS numbers and use, can be found in Table S.2 in **Paper I**. Chemical structures of all substances can be found in Figure 1 (**Thesis**).

1.1.1 Elements

Essential elements

Essential trace elements are crucial for maintaining various physiological functions in the human body. Among these, chromium (Cr), manganese (Mn), cobalt (Co), and selenium (Se) play roles in enzymatic activities, antioxidant defence, and overall metabolic regulation (Jomova et al. 2022; Alexander & Olsen 2023; Kippler & Oskarsson 2024). Understanding their biological significance, sources of exposure, and potential toxic effects is imperative for assessing their impact on human health. Human exposure to essential elements can be biomonitored by measurements in whole blood (Cr, Mn, Co, Se) and serum (Al), assessing the resulting body burden of the elements after long-term exposure.

Chromium is primarily found in its trivalent (Cr(III)) and hexavalent (Cr(VI)) forms. Cr (III) naturally occurs in food, yet its essentiality is disputed. Cr (III) has been postulated to be necessary for glucose metabolism, enhancing insulin action, and lipid metabolism (NDA 2014). In the Swedish Market Basket study 2015, the food group sugar and sweets

contributed most to the total average intake from food, followed by meat products and cereal products (Livsmedelsverket 2017). Cr (III) cause DNA damage in vitro at high doses, but carcinogenicity has not been observed in animal in vivo studies (CONTAM 2014). Industrial processes and contaminated water are common sources of exposure to toxic Cr(VI), which is genotoxic and is classified by the International Agency for Cancer Research (IARC) as a human carcinogen (IARC 2024). Cr is allergenic and may cause contact dermatitis (Alinaghi et al. 2023).

Manganese (Mn) serves as a cofactor for several enzymes involved in energy metabolism, neurotransmitter synthesis, and bone formation (Kippler & Oskarsson 2024). Dietary sources such as cereals and fruits provide essential Mn in Sweden (Livsmedelsverket 2017). High Mn exposure during development is suspected to be a risk factor for neurodevelopmental effects, although conclusions about causality are still uncertain (Kippler & Oskarsson 2024). Very high occupational exposure by inhalation in mining and manufacturing can increase the risk of neurological impairments, highlighting Mn's neurotoxic potential (Karyakina et al. 2022).

Cobalt (Co) functions in vitamin B12 synthesis and is integral to red blood cell formation (FEEDAP 2012). Sugar/sweets and cereals are the primary sources of average cobalt intake in Sweden (Livsmedelsverket 2017). Industrial exposures via battery manufacturing and metal processing may lead to increased risks of respiratory, cardiac, and neurological issues (Leyssens et al. 2017). IARC has classified Co as probably carcinogenic to humans (IARC 2024), and Co may also cause contact dermatitis (Alinaghi et al. 2023).

The intake of selenium (Se) in Sweden, crucial for antioxidant defences and thyroid hormone metabolism (Blomhoff et al. 2023), mainly comes from meat products, followed by fish and cereals (Livsmedelsverket 2017). While essential in small amounts, excessive Se intake can increase the risks of selenosis, characterised by hair and nail brittleness, gastrointestinal disturbances, and neurological impairments (Blomhoff et al. 2023).

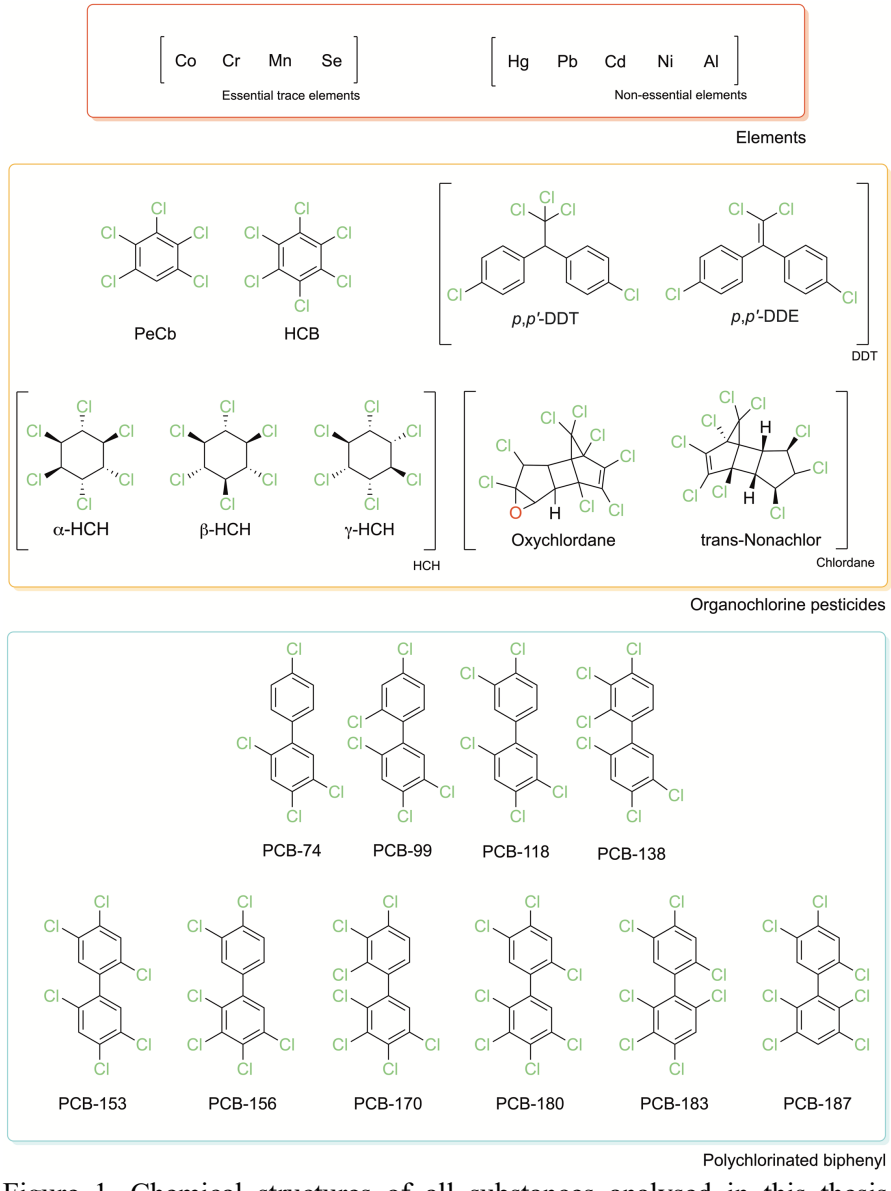
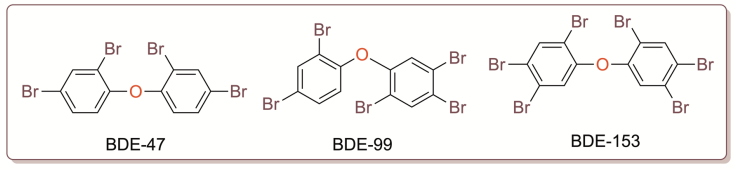
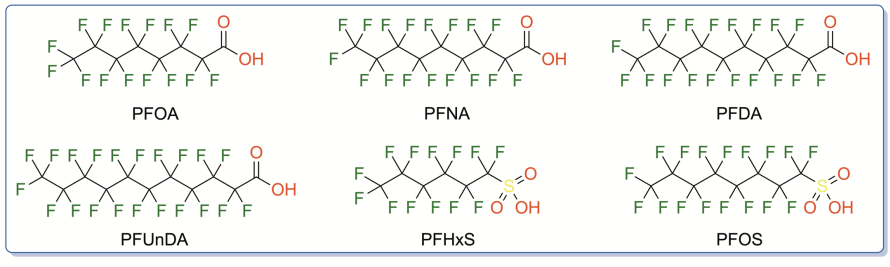


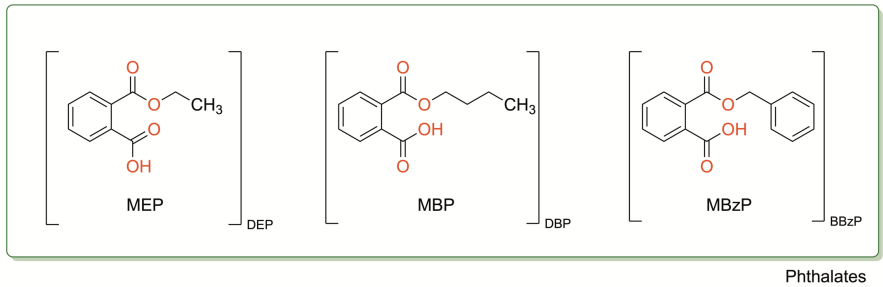
Figure 1. Chemical structures of all substances analysed in this thesis. Brackets signify the substances belonging to a mother compound (bottom-right), with the exception of the elements where it signifies the type of element. Coloured boxes correspond to the substance group (bottom-right).



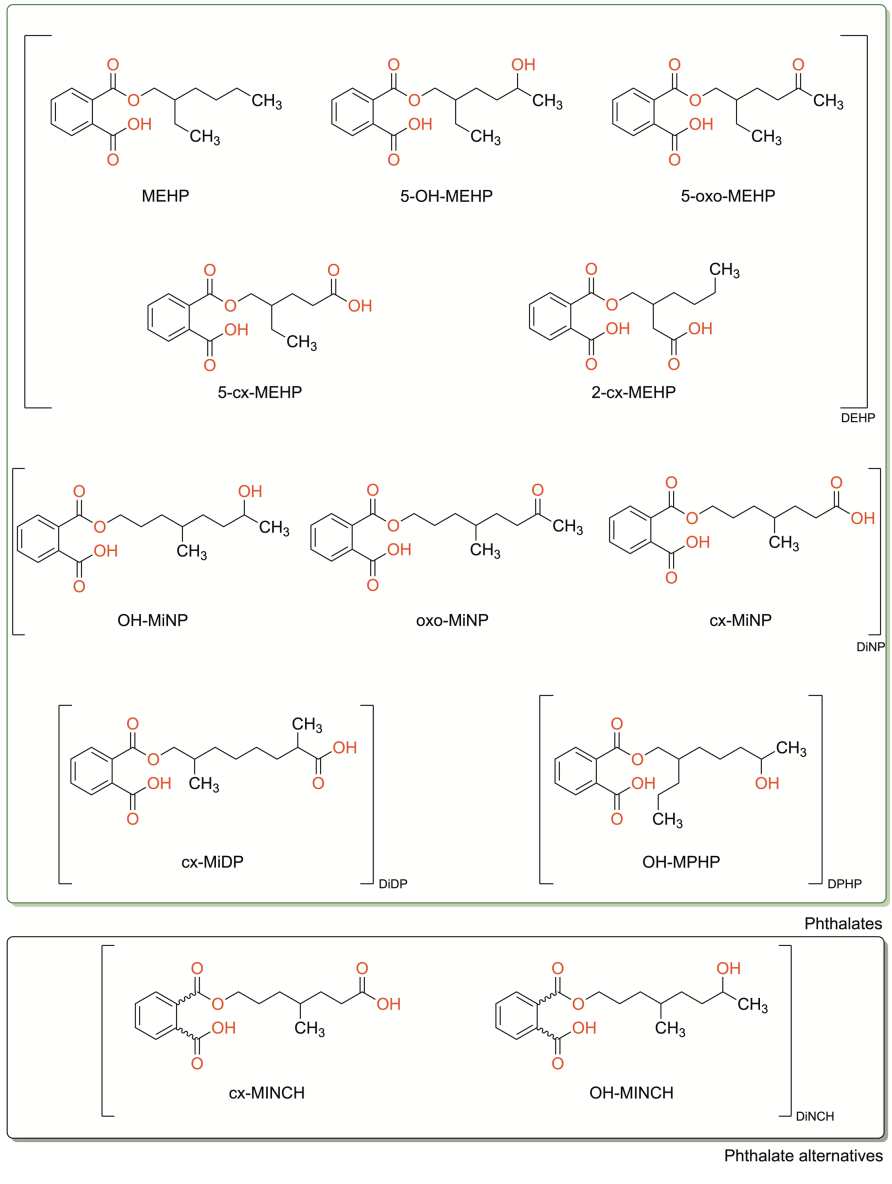
Brominated Flame Retardents

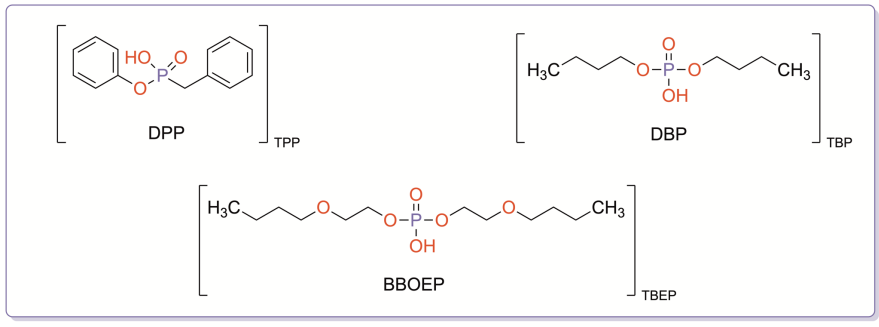


Per- and polyfluoroalkyl substances

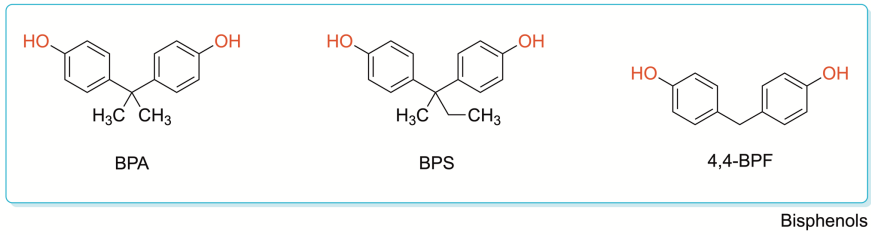


Phthalates

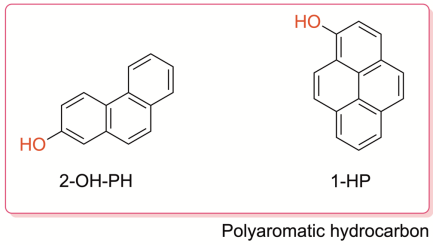

MEHP
5-OH-MEHP
5-oxo-MEHP
5-cx-MEHP
2-cx-MEHP
DEHP
OH-MiNP
oxo-MiNP
cx-MiNP
DiNP
cx-MiDP
DiDP
OH-MPHP
DPHP
Phthalates
cx-MINCH
OH-MINCH
DiNCH
Phthalate alternatives



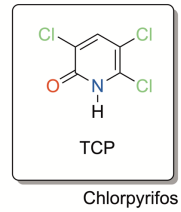
Organophosphate flame retardant



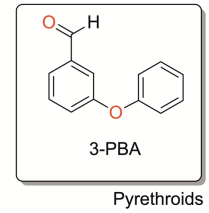
Bisphenols



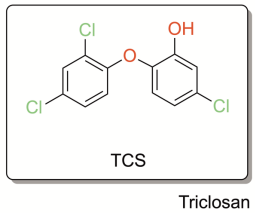
Polyaromatic hydrocarbon



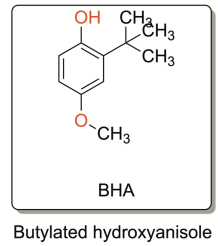
Chlorpyrifos



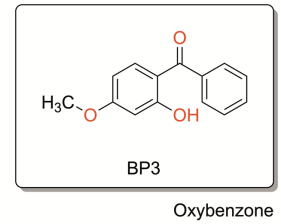
Pyrethroids



Triclosan



Butylated hydroxyanisole



Oxybenzone

Non-essential elements

Non-essential and toxic metallic elements, with significant human exposure from food and, in some instances, drinking water, include nickel (Ni), lead (Pb), mercury (Hg), cadmium (Cd) and aluminium (Al) (Livsmedelsverket 2017). These metals naturally occur in the environment but have become more prevalent in the human environment due to industrial activities such as mining and manufacturing, as well as the use of products such as pesticides and fertilisers (Nordberg et al. 2022).

Sugar/sweets, cereals and fruits are major Ni intake sources in Sweden (Livsmedelsverket 2017). Nickel-sensitised humans have an increased risk of eczematous flare-ups after oral Ni intake (CONTAM et al. 2020b). Ni has been classified as a human carcinogen after inhalation exposure in occupational settings (IARC 2024) and is a risk factor for contact dermatitis (Alinaghi et al. 2023).

Cereals, sugar and sweets, vegetables, fruits and beverages contribute to the intake of Pb in Sweden (Livsmedelsverket 2017). Pb has been linked to increased risks of developmental delays and cognitive impairments in children, as well as kidney and cardio-vascular toxicity in adults (CONTAM 2010). Pb is a probable carcinogen in humans (IARC 2024).

Hg exposure predominantly occurs from fish and seafood in Sweden, mainly in the form of methyl-Hg (Livsmedelsverket 2017). The most sensitive adverse effect of methyl-Hg is related to early life exposure, which is correlated with an increased risk of neuro-developmental effects (CONTAM 2012).

Food is the primary source of Cd exposure in the general population, but smoking can be a significant contributing factor (Garner & Levallois 2016; Ganguly et al. 2018). Cereals, potatoes, vegetables and sugar/sweets are the primary sources of Cd intake from food in Sweden (Livsmedelsverket 2017). Exposure to Cd has been linked to an increased risk of bone demineralisation and kidney dysfunction (EFSA 2009) and is also classified as a human carcinogen (IARC 2024). The primary sources of Al intake in Sweden are sugar/sweets, cereals and fruits (Livsmedelsverket 2017), and adverse effects on reproduction and neurodevelopment are the most sensitive adverse effects found in animal studies (EFSA 2008).

1.1.2 Organochlorine pesticides

Organochlorine pesticides (OCP) are a class of lipid-soluble synthetic chemicals widely used from the 1940s to the 1970s for agricultural and public health purposes mainly due to their effectiveness in controlling a broad spectrum of pests (Carolin et al. 2023). Prominent examples of OCPs include dichlorodiphenyltrichloroethane (DDT), lindane and chlordane, and the fungicide hexachlorobenzene (HCB). These compounds were favoured for their long-lasting residual activity, which reduced the frequency of applications required for pest control. OCP production and use have been severely restricted/banned for decades (UN 2019a). However, their environmental persistence and tendency to bioaccumulate in the food chain have led to widespread contamination and concerns about their toxicological effects on human health (Chopra et al. 2011; Jayaraj et al. 2016). Currently, the primary source of OCP exposure for the general Swedish population is through the consumption of contaminated food, particularly fatty foods such as fish, meat, and dairy products, since food-producing animals accumulate OCPs from the environment (Livsmedelsverket 2017). OCPs are known to be endocrine disruptors, interfering with hormone function, which may lead to increased risks of adverse reproductive and developmental effects (Qi et al. 2022). Lindane is classified as a human carcinogen, DDT and its metabolite p,p'-DDE as a probable human carcinogen, and chlordane as a possible human carcinogen (IARC 2024). OCPs can be biomonitored for long-term human exposure through serum.

1.1.3 PCB

Polychlorinated biphenyls (PCB) are a class of lipid-soluble synthetic organic chemicals comprising 209 different congeners with varying degrees of chlorination. Widely used in the mid-20th century, PCBs were primarily utilised for their chemical stability, heat insulating and non-flammable properties in various industrial applications such as electrical transformers, capacitors, and as additives in paints, sealants, and plastics (UN 2019c). Despite their industrial benefits, the production and use of PCBs have been restricted for decades due to their environmental persistence, bio-accumulative nature, and toxicity (UN 2019a). However, widespread environmental contamination of PCBs and their toxicological impacts on human health are still of concern (Fiedler et al. 2023).

Currently, the primary source of PCB exposure for the general population in Sweden is through the consumption of food, mainly fish, meat, and dairy products (Livsmedelsverket 2017). Chronic exposure to PCBs is associated with increased risks of various adverse health outcomes, including endocrine disruption, neurotoxicity and immunotoxicity (Berghuis & Roze 2019). Furthermore, PCBs have been linked to increased risks of developmental delays and cognitive deficits in children exposed in utero and through breastfeeding (Kimbrough 1995). PCBs can be divided into dioxin-like and non-dioxin-like compounds, and many of the health concerns described previously are mostly related to dioxin-like PCB exposure (DeVito et al. 2024). However, non-dioxin-like PCBs have the same target organs as dioxin-like PCBs, and human exposure to the two substance groups is usually highly correlated, potentially making it difficult to discern dioxin-like and non-dioxin-like effects in humans (Kvalem et al. 2009; Lignell et al. 2011). Dioxin-like PCBs are classified as human carcinogens by the IARC (IARC 2024). Long-term PCB exposure can be biomonitored in serum samples.

1.1.4 PFAS

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of man-made chemicals that have been extensively used in industrial and consumer products since the 1940s. PFAS are popularly known as the "forever chemicals" since many PFAS do not break down in the environment (Ricolfi et al. 2024). Especially perfluorinated alkyl acids (PFAA), which have fully fluorinated alkyl chains that, apart from being produced for specific applications, are also degradation end products of some of the none fully fluorinated poly-fluoroalkyl substances (Gebbink et al. 2015). Known for their resistance to heat and water/oil repelling properties, PFAS are commonly found in products such as non-stick cookware, water-repellent clothing, stain-resistant fabrics, firefighting foams, and various coatings. The widespread use of PFAS, coupled with their environmental persistence, has led to ubiquitous environmental presence and rising concerns about their potential health impacts (CONTAM et al. 2020a). Long-term PFAS exposure may be biomonitored in serum samples.

Human exposure to PFAS can occur through multiple pathways, including ingestion, inhalation, and dermal contact (CONTAM et al. 2020a). In Sweden, fish/seafood consumption is a major source of intake of the long-

chain PFAA perfluoro-nonanoic acid (PFNA), -decanoic acid (PFDA), -undecanoic acid (PFUnDA), and -octanesulfonic acid (PFOS) (Livsmedelsverket 2017). For perfluorooctanoic acid (PFOA), several food groups contribute equally to average intake, and intake of -hexanesulfonic acid (PFHxS) from food is not detectable (Livsmedelsverket 2017). Drinking water contamination could also be a major source of exposure, particularly of PFOS and PFHxS in areas with drinking water sources near industrial sites, fire-fighting training sites at military bases/airports, and wastewater treatment facilities where PFAS have been used or discharged (Hu et al. 2016; Nyström-Kandola et al. 2023). The toxicological effects of PFAS on human health is an active area of research, with mounting evidence linking exposure to increased risks of a variety of adverse health outcomes. PFAS have been associated with increased risks of developmental effects in infants and children, such as low birth weight and immunotoxicity (CONTAM et al. 2020a). They are also linked to increased cholesterol levels, liver enzyme alterations, and disruptions in thyroid hormone levels (CONTAM et al. 2020a). Epidemiological studies have suggested associations between PFOA/PFOS exposure and an elevated risk of certain cancers, including kidney and testicular cancer (Steenland & Winquist 2021). PFOA has been classified as a human carcinogen, and PFOS as a possible human carcinogen (IARC 2024). Production and use of long-chain PFAA and related substances have been phased out during the last decades, yet they have often been replaced by other PFAS with similar environmental and toxicological properties (Ahrens & Bundschuh 2014).

1.1.5 PBDE

Brominated flame retardants (BFR) are a group of chemicals commonly added to various consumer products to inhibit the spread of fire. Among BFRs, lipid-soluble and persistent PBDEs have been used for many decades, but PBDE production and use have now been restricted/banned under the Stockholm Convention (UN 2019b) due to concerns regarding their persistence in the environment, potential for bioaccumulation, and adverse health effects (UN 2019a). Dietary intake is a primary PBDE exposure source, mainly through the consumption of fatty foods such as fish and meat products (Livsmedelsverket 2017). However, due to the release of PBDE from consumer products, household dust can also be an exposure source (Sahlström et al. 2015). The toxic effects of PBDE observed in animal

studies include endocrine disruption and neurodevelopmental and reproductive effects (Gomes et al. 2024). Serum is used for human biomonitoring of long-term PBDE exposure.

1.1.6 Phenols

Phenols, such as bisphenols, TCS, BP-3 and BHA, are a class of aromatic organic compounds characterised by a hydroxyl group (-OH) attached to a benzene ring (Figure 1, **Thesis**). They are widely used in the production of various industrial products, including plastics, resins, adhesives, pharmaceuticals, personal care and household products. Their broad applications make phenols prevalent in both industrial and domestic environments, leading to significant human exposure (CEP et al. 2023; Fandiño-Del-Rio et al. 2024). The general population is exposed to phenols through the consumption of contaminated food, but exposure may also occur through the use of phenol-containing consumer products (CEP et al. 2023; Fandiño-Del-Rio et al. 2024). These phenols with short half-lives are biomonitored in urine samples, and measurements in single samples only represent short-term exposures of hours or days prior to sampling. Bisphenols, TCS, BP-3 and BHA are recognised as endocrine disruptors in animals (Ghazipura et al. 2017; CEP et al. 2023; Zhang et al. 2023a; Hassan et al. 2024). Bisphenol A (BPA) is one of the most extensively studied phenolic substances, showing a plethora of toxic effects in animals (Michałowicz 2014). Immunotoxicity is currently the most sensitive adverse endpoint used in risk assessment of EFSA (CEP et al. 2023).

Triclosan (TCS) is an antimicrobial agent found in various consumer products, including soaps and toothpaste, leading to widespread human exposure and has been linked to increased risks of endocrine disruption, reproductive problems and potential impacts on immune function (Weatherly & Gosse 2017). Butylated hydroxyanisole (BHA) is an antioxidant food preservative used to prevent oxidation, found in fats, oils, cereals, and snacks; it has been linked to increased risks of neurotoxic, endocrine disrupting, reproductive dysfunction and carcinogenic health effects (Zhang et al. 2023a). Benzophenone-3 (BP-3), also known as oxybenzone, is a common ingredient in sunscreens and personal care products, with concerns about causing increased risks of endocrine-disrupting effects and reproductive dysfunction effects, particularly amongst women (Mustieles et al. 2023).

1.1.7 Phthalates

Phthalates are a group of chemical compounds primarily used as plasticisers, which are substances added to plastics to increase their flexibility, transparency, durability, and longevity. Phthalates are used in materials of medical devices, packaging and building materials, personal care products, and toys (Schettler 2006). The extensive use of phthalates in everyday products results in widespread human exposure (Koppen et al. 2019; Sugeng et al. 2020). One major source of exposure to some phthalates is ingesting food and beverages that have been in contact with phthalate-containing materials (CEP et al. 2019). Phthalates leach out of products into the air and dust, leading to inhalation exposure, particularly in indoor environments (Li et al. 2024). Additionally, skin contact with phthalate-containing personal care products can contribute to dermal absorption (Yang et al. 2023). Phthalates are biomonitored as metabolites in urine samples. Due to the rapid metabolism of the mother compounds in the body, the assessed body burdens in single urine samples only represent short-term exposure of a few hours or days before sampling. Phthalates are recognised endocrine disruptors. Experimental studies of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBzP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) have shown anti-androgenic effects causing adverse reproductive effects (CEP et al. 2019).

1.1.8 Phosphorous flame retardants (PFR)

PFRs are organophosphate chemicals used as alternatives to BFRs and also as plasticisers (Wei et al. 2015). Human exposure may occur through air and dust inhalation, but also from food (Wei et al. 2015). In Sweden, concentrations of 8 PFR were analysed in 12 food groups; with 4 having a detection frequency of 30% or higher (Livsmedelsverket 2017). Several different food groups contributed to the intake of the PFRs. For instance, fats/oils contributed significantly to the average triphenylphosphate (TPP) intake, followed by meat products, beverages, and cereals. Tri-n-butylphosphate (TBP) was only detected in a single food group sample, whereas tri(2-butoxyethyl)phosphate (TBEP) could not be detected in any of the food group samples (Livsmedelsverket 2017). PFRs are biomonitored as metabolites in urine, and the measured body burden in single-spot urine samples represents only short-term exposure. Studies of the toxic effects of PFRs in animals are scarce. A risk assessment from 2012 by the Agency for

Toxic Substances and Disease Registry (ATSDR, USA) summarised the animal studies published so far, reporting urinary bladder toxicity as the most sensitive effect of TBP and hepatotoxicity of TBEP (ATSDR 2015).

1.1.9 Polycyclic aromatic hydrocarbons (PAH)

PAHs are produced by the incomplete combustion of organic matter. Common exposure sources include cigarette smoke, air pollution, and food. The food group sugar/sweets contributed the most to the average intake in Sweden, followed by cereals and fats/oils (Livsmedelsverket 2017). PAHs are monitored as metabolites in urine samples, and single spot samples represent short-term exposure. PAH metabolites are known for their potential to cause mutagenic and carcinogenic effects. The PAH benzo[*a*]pyrene is classified as a human carcinogen by IARC, whereas 11 other PAHs are classified as possibly human carcinogens (IARC 2010). PAHs, such as phenanthrene and pyrene, could not be classified by IARC due to a lack of data.

1.1.10 Pesticides – chlorpyrifos and pyrethroids

Chlorpyrifos is an insecticide, with vegetables and fruits as the primary exposure source in Sweden (Widenfalk & Mie 2018). EFSA has reported neurotoxicity as the most sensitive adverse toxic effect in animals, with support from epidemiological studies of children. Moreover, the genotoxic potential could not be excluded (EFSA 2019). Due to health concerns caused by chlorpyrifos intake, this pesticide is no longer approved in the EU (EC 2019). Vegetables and fruits are also major exposure sources of pyrethroids in Sweden (Widenfalk & Mie 2018). Similar to chlorpyrifos, pyrethroids are neurotoxic in animals and are suspected to cause neurodevelopmental effects in children (Elser et al. 2022). These pesticides are biomonitored as metabolites in urine, with single-spot samples representing short-term exposure.

1.2 Adolescents

Adolescence is a critical developmental period characterised by rapid physical, emotional, and cognitive changes. These changes include rapid growth spurts, hormonal fluctuations, and the maturation of vital organ systems (Chulani & Gordon 2014). This period of life may be vulnerable to chemical exposure, potentially disrupting the developmental process and leading to immediate and long-term health consequences (Kiess et al. 2021). Therefore, the investigation of chemical mixture exposure and how chemical mixtures affect adolescents are of paramount importance, encompassing health, developmental, and societal dimensions.

The adolescent brain is particularly susceptible to environmental exposure due to its ongoing maturation processes, including synaptic pruning, myelination, and prefrontal cortex development (Brenhouse & Schwarz 2016). Chemical exposures during this critical period can potentially affect immune maturation, which has been further linked to cognitive deficits, behavioural changes, and mental health disorders (Brenhouse & Schwarz 2016). For example, exposure to neurotoxicants such as lead, mercury, and certain organic solvents has been linked to impairments in attention, memory, and executive function (Grandjean & Landrigan 2014).

Additionally, adolescence is a period of heightened vulnerability to stress, with long-lasting implications for mental health. Chemical exposures that disrupt endocrine and immune system functioning can exacerbate stress responses, increasing the risk of psychiatric disorders such as depression (Brenhouse & Schwarz 2016).

Although recent efforts have been made to increase the biomonitoring of chemical exposure of adolescents separately from children (Govarts et al. 2023), research into the health effects of chemical mixtures on adolescent populations remains relatively sparse compared to those focusing on either children or adults.

Investigating the effects of chemical mixtures on adolescents also has significant societal and policy implications. Adolescents represent the future, whether from a humanitarian or practical perspective, as they are the next generation of workforce and societal contributors; thus, their health and well-being will likely directly influence future societal outcomes.

1.3 Toxic effects of mixtures

Current legislation on chemicals often focuses on individual chemicals, overlooking the potential risks of chemical mixtures. Comprehensive research can provide the evidence needed to implement more holistic regulatory approaches that account for the complexities of chemical interactions (Kortenkamp et al. 2007). Yet most in-vitro (cell) and in-vivo (animal) research into the toxicity of chemical mixtures have typically involved either simple mixtures or only one substance group (e.g., PFASs, PCBs) (Bopp et al. 2019). From a regulatory and risk assessment standpoint, research into chemical mixture exposure and toxicity of complex chemical mixtures are of vital importance, as the possibility of combined interactions can result in unpredictable and often more severe toxicological outcomes than those observed with single compounds (Braun et al. 2016). Unlike single chemicals, which have been extensively studied for their individual toxicological profiles, substances in mixtures can together cause synergistic, antagonistic, or additive effects, leading to enhanced toxicity or novel toxic effects (Bopp et al. 2019). This complexity complicates risk assessment and regulatory standards, as traditional toxicological evaluations may not adequately capture the hazards presented by chemical mixtures.

1.3.1 Investigating chemical mixtures in epidemiological studies

Compared to experimental in-vitro and in-vivo studies, analysing potential chemical mixture effects in an epidemiological study on humans presents significant methodological and interpretative challenges. One of the primary difficulties is the complexity of the mixtures themselves. Environmental exposures often involve numerous chemicals/chemical groups simultaneously with a high degree of correlation between some substances, such as between the congeners of PCB (DeVoto et al. 1997). This leads to multicollinearity problems in the statistical analyses, whereby the standard errors of the regression coefficients become drastically inflated (Allen 1997). Moreover, including large numbers of chemicals and many covariates in the analyses may cause overfitting where the regression fits the ‘noise’ rather than the actual underlying relationship (Rohloff et al. 2023). Additionally, substances in the mixture potentially have varying metabolic pathways and degrees of toxicity, which may lead to non-linear effects and potential for interactions. As a result, traditional epidemiological regression methods, which typically focus on single-exposure models, are insufficient for

capturing the multifaceted nature of chemical mixtures (Bobb et al. 2015). Advanced statistical techniques, such as weighted quantile sum regression, quantile g-comp and Bayesian kernel machine regression (BKMR) (Bobb et al. 2015; Carrico et al. 2015; Keil et al. 2020), have been developed to address these complexities. However, these methods require large datasets and substantial computational power, and they often necessitate assumptions that may not fully capture real-world exposure scenarios. Yet, of these methods, BKMR shows the most promise for better handling the aforementioned problems (Hoskovec et al. 2021).

1.3.2 Non-linear effects

There is the potential for the dose-response relationship to be non-linear; for example, PFNA at low and high body burdens may increase the odds of women's infertility, but median body burdens decrease the same effect-response, creating a 'U' shape coefficient curve (Hill et al. 2018; Tan et al. 2022). Therefore, it is crucial that the statistical modelling used for analysing complex chemical mixtures in epidemiology can estimate these types of relationships, as it would be conservative to assume that the probability of non-linear dose-effect relations on the outcome increases as the number of substances and complexity of the chemical mixture increases. However, interpretation of the results for non-linear analyses in epidemiology and its links to mechanistic knowledge remains challenging, whilst also being difficult to communicate cohesively to risk assessors and risk managers.

1.3.3 Additive effects and interactions

In the context of toxicology, mixture effects can generally be assumed to be non-interactive, whereby chemicals with the same mode of action in the mixture typically show additive effects, typically referred to as dose addition/concentration addition (Martin et al. 2021). The term *interactions* in a mixture exposure context would mean a divergence of this additive assumption for chemicals with the same mode of action, whereby the concentration of one substance alters the toxic effect of another. This can be referred to as *synergistic*, where the toxic effect of the mixture is higher than additive, or *antagonistic*, where the toxic effect is lower than additive. A special case is when a substance alters the toxic effect of another substance synergistically or antagonistically without inducing a toxic effect itself, which can be referred to as an *effect enhancer* (Martin et al. 2021). Many

traditional statistical methods have been unable to assess such interactions within epidemiological studies of chemical mixtures. Of the few existing methods, interpretability and communication of the results remain difficult with no 'gold standard' currently established.

1.4 Risk assessment of chemicals

The risk assessment process for toxic chemicals is a systematic approach used to evaluate the potential health risks posed by human exposure to hazardous substances. This process is fundamental in evaluating chemical effects on human health and regulatory decision-making. Risk assessment typically comprises four main steps. Each step contributes critical information to the overall understanding of the health risks and helps inform appropriate risk management actions (WHO 2009a). In the case of chemical mixtures, another level of complexity needs to be addressed where health outcomes or risk can potentially be cumulatively realised by a mixture of chemicals compared to when analysing the risk of single substances separately.

The four main components that encompass the human health risk assessment process for toxic substances are as follows (WHO 2009a):

I. **Hazard identification** entails identifying substances suspected to pose health hazards (e.g., neurotoxic, carcinogenic, endocrine disruption) and identifying the possibility of human exposure.
II. **Hazard characterisation** focuses on evaluating the quantitative relation between the exposure dose of the substances and adverse health effects, sometimes referred to as the exposure-response effect. Also, to identify the adverse effects that occur at the lowest exposure levels (i.e., critical effects) and the safe level of exposure of humans if possible (health-based guidance values, HBGV).
III. **Exposure assessment** involves identifying population groups at risk of exposure and quantifying the magnitude, frequency, duration and routes of exposure of said groups.

IV. **Risk characterisation** is the end point of the risk assessment process, where evidence gathered from the previous steps is assessed to determine the health risk of the exposed population. This is often done by comparing the exposure levels in different populations with the HBGV. If the exposure levels in a population are below the HBGV, then the exposure is not regarded as a health concern. If the exposure of some parts of the population is above the HBGV, then it is considered a health concern.

2. Thesis aims & overview

This thesis will examine chemical mixture exposure in a Swedish adolescent study population (Riksmaten Adolescents 2016-17; RMA), identifying potentially problematic exposure levels. We will also explore socio-demographic determinants within the study population and exposure differences among demographic sub-groups. Additionally, we will investigate whether single or combined mixture effects have adverse health outcomes, particularly in the context of blood serum vaccination antibody titres. The main objective is to conduct a comprehensive assessment of the exposure of Swedish adolescents to the chemical mixture in question and to generate good data on the potential immune-modulating effects of the mixture.

2.1 Aims

The objectives of this thesis can be summarised in the following points:

I. Explore the body burdens of the RMA participants of a diverse chemical mixture and compare biomonitored substance concentration levels to those found in other similar adolescent populations.

II. Identify if any substance concentration levels exceed published HBGVs, as well as take a first look at potential health concerns caused by combined exposure to compounds with the same target organ of toxicity.

III. Examine the association of substances in the mixture to various socio-economic determinants, thus identifying risk groups of higher exposures.

IV. Evaluate the total mixture effect on the immune-health endpoint of vaccine antibody titres. Explore the contribution of single compound to potential changes in immune health and if there are any interactions between substances within the mixture in relation to the health outcomes.

2.2 Risk assessment overview

The following subsections provide a brief overview of this thesis's three papers, their main research aim, and how they fit within the risk assessment framework.

2.2.1 Hazard identification

Paper I examined the concentration levels of the analysed chemicals in whole blood, serum or urine in the RMA adolescent population group, thus identifying which chemicals could be detected and which could not be detected.

2.2.2 Hazard characterisation

The association between body burdens of the chemical mixture and a potentially sensitive adverse effect, in this case, vaccination antibody titres among the RMA participants, were studied in **Paper III**. The aim was to identify overall mixture effects, univariate dose-response relationships and potential interactions. **Paper III** focused on a subset of the study population from **Papers I & II**, which had complete data available for the analysed elements and POPs, blood serum concentrations of vaccination antibodies, and demographic and lifestyle covariates. We examined vaccination antibodies against diphtheria, tetanus and pertussis toxoids (DTP), and also against measles and rubella viruses (MR). In this study, the rapidly eliminated substances in urine were excluded since only single-spot urine samples were taken, making it impossible to determine more long-term exposure. Several urine samples across a long period need to be taken to estimate the typical long-term exposure rate of the participants (Roggeman et al. 2022). The estimation of only short-term exposure with a urine single-spot sample would diminish the confidence in any associations to immunomodulating effects if identified.

2.2.3 Exposure assessment

We reported the individual substances' body burdens and exposure profiles within the assessed mixture in **Paper I**. In **Paper II**, associations between socio-demographic determinants and substance concentrations were evaluated. The main determinants examined were gender, age, participant/maternal birth, country income per capita level, parental education levels, and geographic place of living (longitude/latitude). This study investigated if there were demographic differences in body burdens of different substances/substance groups and identified population sub-groups that were at risk of having higher exposures. Additionally, other non-demographic determinants were used in subsequent auxiliary analysis to see if any demographic associations could be explained by known routes of exposure found in different populations from Sweden or by lifestyle factors known to influence exposure.

2.2.4 Risk characterisation

In **Paper I**, we compared the detected concentrations of substances with published human biomonitoring guidance values (HBM-GV) and quantified the proportion of participants with concentrations above HBM-GVs. Additionally, we performed a Hazard Index (HI) analysis where we identified three main target organs for critical effects common to many of the substances examined (assuming additive effects) and estimated the proportion of the study population at risk of having an HI that would indicate a health concern.

3. Methods

This section provides an overview of the various methods used throughout all the papers included in this thesis. More in-depth details can be found in the subsequent methods section of the papers.

3.1 Riksmaten adolescents 2016-17 (RMA)

RMA is a nationally representative cross-sectional survey conducted by the Swedish National Food Agency (SFA) to evaluate the dietary habits of school-attending adolescents across Sweden (Moraeus et al. 2018). The survey was not just limited to food consumption but also included a biomonitoring part assessing body burdens of elements, POPs and substances rapidly eliminated in urine, as well as various metadata such as socio-demography, lifestyle habits and certain physiological factors. The population was based on school grades 5 (mean age 11.5), 8 (mean age 14.5) and 11 (mean age 17.7).

In total, 619 schools were chosen by Statistics Sweden to be invited to join the study, roughly with 200 for each school grade. As schools and grades were selected to be representative of the adolescent school population across Sweden, the criteria for invitations took into account whether the schools were public or private and geographical spread. Schools with <10 students or with only language education were excluded. Of the 619 schools chosen, 40% (N=259) were randomly invited to participate with biomonitoring sampling, of which 62 schools agreed to participate, totalling to 1305 participants (Figure 2, **Thesis**). From these 1305 participants, we created subsets for **Papers I, II & III** depending on the requirements of the statistical modelling, i.e., if the participants had complete or incomplete data (Figure

2, **Thesis**). For a visualisation of the spatial density spread of the participants, please see Figure 1 in **Paper II**.

During school visits, participants would complete questionnaires on dietary and lifestyle habits via a web-based method (RiksmatenFlex). Around 97% of participants had access to the internet and an appropriate interface (e.g., smartphone, tablet, computer). Still, for those who did not, a computer or tablet was lent to the participant to complete the questionnaires. Participants who were invited to provide bio-samples were offered 300 SEK in the form of gift vouchers for full participation.

RiksmatenFlex is a website divided into two sections: RiksmatenFlexDiet, which the participants use to register dietary intake of repeated 24-hr recalls and RikmatenFlexQ, which is the lifestyle questionnaire part also filled in by the participants. In RiksmatenFlexDiet, three 24-hour recalls were registered, with the first recall day always being the day before the school visit, the second being the day of the visit, and the third being randomly assigned 2-7 days after the school visit, but always on a weekday. Access to the survey was only granted on the assigned days and could not be pre-emptively filled. The spread of school visit days was planned in order to get a representation of all weekdays. Food was recorded as one of 778 generic food items that could be selected from the survey; after this, further information could be added in a secondary step, resulting in over 2300 unique food items. The food items were then referenced in the SFA food composition database to automatically calculate energy and nutrient intakes (i.e., habitual intakes), used in **Papers II & III**. Participants could specify the proportion of food they ate and were given picture guides to help calculate the portions.

RiksmatenFlexQ was divided into four questionnaires: ‘You and the family’, ‘Eating habits’, ‘More eating habits’, and ‘Parental questionnaire’. Frequency questions on food consumption were included in ‘Eating habits’ and ‘More eating habits’, which covered food frequencies used in **Paper II**. The ' You and the family ' questionnaire included physical activity and characteristics of the child and parents. The ‘Parental questionnaire’ included questions intended for the parents to fill in as it would likely be difficult for the adolescents to answer themselves, for instance, including questions on months spent breastfeeding the study participant after birth.

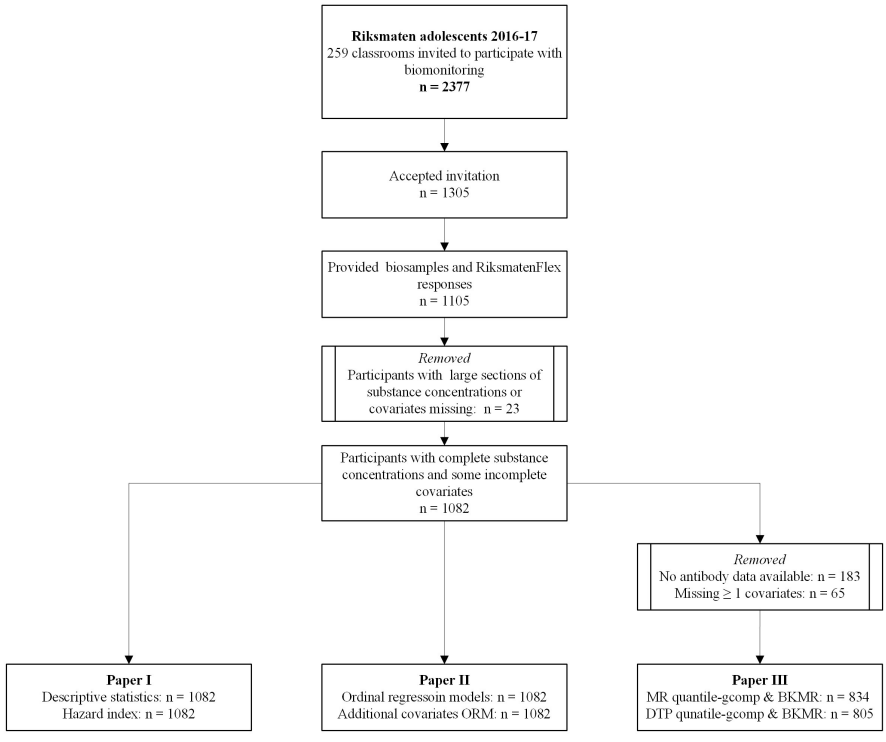


Figure 2. Flowchart of how the number of participants from the RMA survey were selected for **Papers I, II & III**.

3.2 Sampling & chemical analysis

In-depth details can be found in the method sections of **Papers I, II & III** and Moraeus et al. (2018). The following subsections will contain a brief amalgamated description of the combined methods.

3.2.1 Sample collection and transportation

Biological sampling was performed by the regional divisions of Occupational and Environmental Medicine (AAM) when visiting the participating school classes. School visits were planned in collaboration with the SFA, and non-fasting blood and urine samples were collected at the school health facilities by the AAM team consisting of 3-4 personnel. Whole blood samples for serum and plasma collection were centrifuged immediately, pipetted into aliquots on site, and placed in freezers along with

urine samples collected simultaneously before being transported to the AMM division office and stored at -80°C. Once the regional AMM division was finished with all school visits of the region, the samples were then transported frozen to the SFA in Uppsala and stored at -80°C.

3.2.2 Blood analysis

Elements (excluding Al) were measured in whole blood samples. Please see section 2.5 of **Paper I** and references (Barany 1997) for details on the complete methods. In brief, whole blood samples were analysed in duplicates, and concentrations were determined using inductively coupled plasma mass spectrometry.

3.2.3 Serum analysis

The POPs, including PCBs, OCPs, PBDEs and PFAS, as well as Al were analysed in blood serum samples. Please see sections 2.6 and 2.7 of **Paper I** and references (Powley et al. 2005; Gasull et al. 2019) for details on the complete methods. Briefly, PCBs, OCPs, PBDEs had concentrations determined using gas chromatography – triple quadrupole mass spectrometry (GC-MS/MS). For PFAS, concentrations were measured using an Acquity ultra-performance liquid chromatograph coupled to a Waters Xevo TQS triple quadrupole mass spectrometer operated in negative electrospray ionisation.

3.2.4 Urine analysis

Phenols, phthalates and substances measured in single-spot urine samples had concentrations measured using liquid chromatography triple quadrupole mass spectrometry. For complete method details, please see section 2.8 of **Paper I** and references (Cequier et al. 2014; Alhamdow et al. 2017; Liljedahl et al. 2021).

Table 1. Count and percentage for each individual country included in the 'participant | maternal birth country per capita income' determinant, separated by the participant and mother. Income is based on the World Bank Group 2018 classification. Total N = 1082.

Country	Income level	Participant N(%)	Maternal N(%)
Afghanistan	Low	10 (0.92 %)	12 (1.11 %)
DR Congo	Low	1 (0.09 %)	1 (0.09 %)
Eritrea	Low	3 (0.28 %)	5 (0.46 %)
Ethiopia	Low	1 (0.09 %)	3 (0.28 %)
Somalia	Low	2 (0.18 %)	10 (0.92 %)
Syria	Low	15 (1.39 %)	18 (1.66 %)
Tanzania	Low	0	1 (0.09 %)
Uganda	Low	1 (0.09 %)	1 (0.09 %)
Yemen	Low	2 (0.18 %)	1 (0.09 %)
Bangladesh	Lower-middle	0	1 (0.09 %)
Bolivia	Lower-middle	0	1 (0.09 %)
Egypt	Lower-middle	1 (0.09 %)	1 (0.09 %)
India	Lower-middle	1 (0.09 %)	1 (0.09 %)
Kenya	Lower-middle	3 (0.28 %)	0
Laos	Lower-middle	0	1 (0.09 %)
Morocco	Lower-middle	0	1 (0.09 %)
Myanmar	Lower-middle	0	1 (0.09 %)
Pakistan	Lower-middle	0	1 (0.09 %)
Philippines	Lower-middle	0	1 (0.09 %)
Senegal	Lower-middle	0	1 (0.09 %)
Sudan	Lower-middle	0	1 (0.09 %)
Tunisia	Lower-middle	0	2 (0.18 %)
Ukraine	Lower-middle	2 (0.18 %)	2 (0.18 %)
Uzbekistan	Lower-middle	2 (0.18 %)	1 (0.09 %)
Vietnam	Lower-middle	0	2 (0.18 %)
Zimbabwe	Lower-middle	1 (0.09 %)	1 (0.09 %)
Albania	Upper-middle	1 (0.09 %)	2 (0.18 %)
Argentina	Upper-middle	1 (0.09 %)	1 (0.09 %)
Armenia	Upper-middle	0	1 (0.09 %)
Azerbaijan	Upper-middle	1 (0.09 %)	0
BosniaHerzegovina	Upper-middle	2 (0.18 %)	16 (1.48 %)
Brazil	Upper-middle	2 (0.18 %)	1 (0.09 %)
Bulgaria	Upper-middle	0	1 (0.09 %)

China	Upper-middle	3 (0.28 %)	2 (0.18 %)
Colombia	Upper-middle	1 (0.09 %)	4 (0.37 %)
Costa Rica	Upper-middle	1 (0.09 %)	0
Iran	Upper-middle	3 (0.28 %)	10 (0.92 %)
Iraq	Upper-middle	14 (1.29 %)	23 (2.13 %)
Kosovo	Upper-middle	0	6 (0.55 %)
Lebanon	Upper-middle	2 (0.18 %)	5 (0.46 %)
Libya	Upper-middle	1 (0.09 %)	0
Montenegro	Upper-middle	0	1 (0.09 %)
North Macedonia	Upper-middle	0	4 (0.37 %)
Peru	Upper-middle	0	2 (0.18 %)
Romania	Upper-middle	1 (0.09 %)	3 (0.28 %)
Russia	Upper-middle	1 (0.09 %)	3 (0.28 %)
Serbia	Upper-middle	0	3 (0.28 %)
Sri Lanka	Upper-middle	0	1 (0.09 %)
Thailand	Upper-middle	6 (0.55 %)	10 (0.92 %)
Turkey	Upper-middle	5 (0.46 %)	16 (1.49 %)
Venezuela	Upper-middle	0	1 (0.09 %)
Chile	High	0	1 (0.09 %)
Croatia	High	0	1 (0.09 %)
Czech Republic	High	0	1 (0.09 %)
Denmark	High	1 (0.09 %)	3 (0.28 %)
Finland	High	0	12 (1.11 %)
Germany	High	4 (0.37 %)	4 (0.37 %)
Hungary	High	1 (0.09 %)	3 (0.28 %)
Iceland	High	0	1 (0.09 %)
Ireland	High	0	1 (0.09 %)
Israel	High	2 (0.18 %)	2 (0.18 %)
Italy	High	1 (0.09 %)	1 (0.09 %)
Japan	High	0	1 (0.09 %)
Latvia	High	1 (0.09 %)	1 (0.09 %)
Netherlands	High	0	2 (0.18 %)
Norway	High	1 (0.09 %)	5 (0.46 %)
Poland	High	4 (0.37 %)	10 (0.92 %)
Portugal	High	0	1 (0.09 %)
Saudi Arabia	High	1 (0.09 %)	1 (0.09 %)
Singapore	High	1 (0.09 %)	0

South Korea	High	1 (0.09 %)	0
Spain	High	2 (0.18 %)	0
Sweden	High	962 (88.91 %)	832 (76.89 %)
Switzerland	High	2 (0.18 %)	0
Taiwan	High	1 (0.09 %)	0
United Kingdom	High	4 (0.37 %)	2 (0.18 %)
United States	High	1 (0.09 %)	4 (0.37 %)
Unknown	Unknown	1 (0.09 %)	6 (0.55 %)

3.3 Data wrangling

3.3.1 Participant/maternal birth country income

A main potential confounding covariate we included in all of the models for **Papers II and III** was the income per capita level of the participants and mothers country of birth. Criteria for the classifications were based on the gross national income per capita of a country, curated by the World Bank Group (WBG 2018). Data for when a participant or their mother arrived in Sweden for foreign-born participants were unavailable. Therefore, we defaulted to the classifications provided for the year 2018 (WBG 2018), which coincides with the last year of sampling for the RMA population. A detailed accounting of the countries for both the participant and mother can be found in Table 1 (**Thesis**). As there were too few participants (N=10) for the original 'lower-middle' income levels (Table 1), we merged the level to the 'low' income level. The participant and maternal birth country per capita income variables were significantly correlated (spearman $\rho = 0.63$), increasing the risk of multicollinearity being introduced to the models. Yet, we deemed that both the participant and maternal birth country income levels were important confounders individually. Therefore, we opted to combine the variables to retain as much of the data in the models as possible whilst circumventing the multicollinearity concern. We decided against including the paternal birth country per capita income level as it was highly correlated to the maternal birth country per capita income level (spearman $\rho = 0.82$), and we did not believe it to share the same confounding effect on both the exposure and the outcome as the maternal variable. The combined levels of the new 'participant | maternal birth country per capita income' variable

became: ‘Low | Low’, ‘High | Low’, ‘Upper-middle | Upper-middle’, ‘High | Upper-middle, ‘High | High’ (Table 1, **Paper II**).

3.3.2 Lifestyle & physiological factors

Gender was included in all papers, but it should be noted that this variable was self-reported in RiksmatenFlexQ via the prompt “Are you a boy or a girl?” and does not represent assigned sex at birth/sampling, for which no data was collected.

As adolescents are not yet adults, standard BMI would be inappropriate; therefore, we used the international obesity task force (IOTF) classification, which has slightly different BMI cut-offs depending on age. We mapped these adjusted cut-offs to our participants, which were divided into the following groups: Severe Thinness, Thinness, Healthy, Overweight, Obese, and Severely Obese. However, some of these classifications had too few numbers, and we decided to merge ‘severe thinness’ and ‘thinness’ to ‘underweight’ and also ‘obese’ and ‘severely obese’ to ‘obese’. This variable was used as a covariate for the models in **Papers II & III**.

Data on smoking and alcohol consumption was collected only from 8th and 11th graders but not 5th graders using the prompts “current smoking habits?” and “alcohol consumption the last six months?”. We assumed that 5th graders did not smoke or drink due to their young age. Initially, data collected were divided into the categories: ‘never’, ‘no, quit’, ‘yes, occasionally’ and ‘yes, daily’ for smoking and ‘no’, ‘yes, once’, and ‘yes’ for alcohol. Due to low numbers in the latter two categories for smoking specifically, we merged ‘yes, occasionally’ and ‘yes, daily’ to ‘yes’. Smoking and alcohol consumption were used as a covariate in models included in **Papers II & III**.

Maternal and paternal education levels were included in the main models of **Paper II**. Data was self-reported and collected via RiksmatenFlexQ using multiple choice questions. No further changes were made to the variables, each having four levels: “Elementary school or none”, “2 years of upper-secondary or vocational”, “3 years+ of upper-secondary or vocational”, and “Higher education (University or equivalent)”.

Physical activity was only included in **Paper III** as we believed it would affect the outcome variable of vaccine antibody concentrations, as described in the direct acrylic graph (Fig. S.1, **Paper III**). This data was self-reported and collected via RiksmatenFlexQ, with the prompt asking how active they

have been in the past week and giving four possible answers with several examples of relevant exercises per answer. These answers were then translated to the activity levels: 'sedentary', 'light', 'moderate', and 'rigorous'.

Breastfeeding was included in some of the additional covariate models in **Paper II** and all of the BKMR and quantile-gcomp models in **Paper III**. Breastfeeding is a known exposure source for some POPs and is linked to the immune development of children (Table S.1, **Paper II**; Methods, **Paper III**) (M'Rabet et al. 2008). In **Paper II**, the categorical variable was labelled 'Nursing' and was coded as: 'Never', '<1 month', '1-3 months', '4-6 months', '7-12 months', and '>12 months'. In **Paper III**, the variable was coded numerically as the months the participant was breastfed.

Season of sampling was included in all the additional covariate models in **Paper II**. Evidence from the literature suggests that exposure levels for many substances are sensitive to the time of sampling (Table S.1, **Paper II**). The data was originally coded as month of sampling, but changed to seasons due to many months lacking sufficient numbers. December, January, and February were coded as 'Winter'; March, April, and May as ' Spring'; and September, October, and November as 'Autumn' (Table S.1, **Paper II**). None of the students were sampled during the summer months.

3.3.3 Food consumption

In **Papers II & III**, we used long-term consumpton of food groups consumed regularly by the participants. This was split into habitual intake (numerical g/day) and consumption frequencies (categorical). Data for habitual intake was gathered from the two independent 24-hour recall dietary registrations in RiksmatenFlexDiet and calculated into grams per day (Methods, **Paper II & III**). Data on food frequencies used in **Paper II** were taken from RiksmatenFlexQ.

3.3.4 Substance concentrations below limits of detection

In **Papers I, II & III** we used machine reads for all substances below limits of detection (<LOD) or limit of quantification (LOQ), meaning the value provided by the measuring instrumentation even if it was <LOD or <LOQ. As explained in both papers, this was done in lieu of a single point of conversion, such as by transforming <LOD to LOD/√2, as is typically done in many studies (Croghan & Egeghy 2003), in an attempt to reduce bias

(AMC 2001; Bergstrand & Karlsson 2009). An example of the distribution of machine read vs LOD/√2 can be seen in Supplementary Figure 1, **Paper I**. The main advantage this solution offers is that it provides a broader distribution of <LOD data rather than a single point value, which can significantly bias many statistical models. The main drawback of using machine reads is that these values are less certain than those above LOD or LOQ. The only exception was PFAS values <LOD, which the analytical laboratory deemed too uncertain. In **Paper II**, machine reads would sometimes be presented as 0 (i.e., unmeasurable by the instrument); these values, along with the PFAS values <LOD, were converted to 0.0001. This number was selected as it was lower than any concentration value regardless of the unit of concentration, and it would help satisfy the assumption that it is improbable that any sample would have absolutely no trace of the analysed substance but rather an amount which is not measurable. For **Paper III**, we again decided to use the machine reads; however, in the cases where values were registered as 0, as it was required to log scale the components of the mixture for the BKMR analysis, we opted to convert any 0 values to 'non-zero minimum'/2 before natural log converting. Table 2 briefly overviews the count and percentage of substances recorded initially as 0.

Table 2. Count and percentage of participants with substance values that were recorded as 0, meaning unmeasurable by the analytics instrument. Substances not analysed in **Paper III** were marked (–).

Substance	Papers I & II (N=1082)	Paper III (N=834)
Cr	0	0
Mn	0	0
Co	1 (0.09%)	0
Ni	1 (0.09%)	0
Se	0	0
Cd	0	0
Hg	1 (0.09%)	0
Pb	0	0
Al	0	0
PeCB	192 (17.7%)	158 (18.9%)
HCB	0	0
α–HCH	731 (67.6%)	–
β–HCH	9 (0.83%)	8 (0.96%)
γ–HCH	708 (65.4%)	–
Oxychlordane	588 (54.3%)	–
trans-Nonachlor	0	0
p,p'-DDT	524 (48.4%)	–
p,p'-DDE	0	0
PCB-74	0	0
PCB-99	0	0
PCB-118	0	0
PCB-138	0	0
PCB-153	0	0
PCB-156	1 (0.09%)	0
PCB-170	0	0
PCB-180	0	0
PCB-183	1 (0.09%)	0
PCB-187	0	0
BDE-47	137 (12.7%)	103 (12.4%)
BDE-99	262 (24.2%)	201 (24%)
BDE-153	87 (8%)	64 (7.7%)
L-PFOA	1 (0.09%)	1 (0.12%)
PFNA	53 (4.9%)	36 (4.3%)

PFDA	216 (20%)	158 (18.9%)
PFUnDA	332 (30.7%)	–
L-PFHxS	7 (0.6%)	6 (0.72%)
L-PFOS	0	0
br-PFOS	0	0
MEP	0	–
MBP	0	–
MBzP	0	–
MEHP	0	–
5-OH-MEHP	0	–
5-oxo-MEHP	0	–
5-cx-MEPP	0	–
2-cx-MEHP	0	–
OH-MiNP	0	–
oxo-MiNP	0	–
cx-MiNP	0	–
cx-MiDP	0	–
OH-MPHP	0	–
oxo-MINCH	0	–
cx-MINCH	1 (0.09%)	–
OH-MINCH	0	–
DPP	0	–
DBP	1 (0.09%)	–
BBOEP	0	–
BPA	5 (0.46%)	–
BPS	0	–
4,4-BPF	22 (2%)	–
2-OH-PH	0	–
1-HP	14 (1.3%)	–
TCP	0	–
3-PBA	0	–
TCS	6 (0.55%)	–
BHA	28 (2.6%)	–
BP-3	0	–

3.4 Statistics

This thesis used R as the primary programming language for all the data cleaning, modelling and figure presentations. **Papers I & II** used R core version 4.0.5, whilst **Paper III** used core version 4.3.1. Multiple imputations were implemented in **Paper II** using the 'mice' R package (ver. 3.10.0). Additionally, in **Paper II**, Ordinal regression models were implemented using the 'rms' R package (ver. 6.0-0), with the multiple imputations fitted to the models via functions taken from the 'Hmisc' R package (ver. 4.4-0). The Bayesian kernel machine regression (BKMR) and quantile g-comp models were implemented using the 'bkmr' R package (ver. 0.3.3) and 'qgcomp' R package (ver. 2.15.2) for the analysis in **Paper III**.

In-depth details of the statistical methods and implementation can be found in the method sections of **Papers I, II & III**. However, the following sections provide a brief overview and elaborates further on the missing context of the methods if not previously covered.

3.4.1 Multiple imputation

In **Paper II**, we used multiple imputations by chain equations (MICE) to deal with data we assumed to be missing at random (Buuren & Groothuis-Oudshoorn 2011). As described in the methods section of **Paper II**, using Rubin's rules, we fitted 16 multiple imputations and 5 iterations to the ordinal regression models, thereby correctly adjusting estimates and standard errors.

We decided not to use MICE for the BKMR or quantile g-comp models in **Paper III** as there is no readily available implementation to incorporate the multiple imputations correctly using Rubin's rules into the models; hence, we opted for using complete cases instead. In this context, complete cases mean that participants included in **Papers I & II** were only kept in **Paper III** if they had data available for all substance concentrations and covariates included in the BKMR and quantile g-comp models (Fig. 2, **Thesis**), a complete list of which can be found in the methods section of **Paper III**.

3.4.2 Hazard ratio & hazard index

Hazard ratios (HR) represent the ratio between the measured concentration of a single substance in each participant and previously published HBM-GV

concentrations of the same substance (Borg et al. 2013). The hazard index (HI) of a participating adolescent is the sum of the HRs for all substances with the same critical target organ used for determining the HBM-GVs (Methods, **Paper I**). The HI method assumes an additive effect of all substances included in a critical target organ group, and also assumes the same mode of action of these substances on the target organ, which in this case was the liver, kidney and central nervous system (CNS). The mode of action assumption is very conservative since it is not likely that many of the substances included in the HI calculations have the same mode of action. Therefore, The HI results are likely to overestimate the health concern and should be regarded as a first assessment of possible mixture health risks for further investigation in the future.

3.4.3 Ordinal regression models

Ordinal regression models (ORM) are typically used to model ordinal-level dependent variables with a distinctive hierarchy or order. In **Paper II**, we specifically used cumulative probability models, a type of ORM. A basic example of an ordinal variable would be the physical activity variable, as seen in section 2.3.2, which follows the order 'sedentary' < 'light' < 'moderate' < 'rigours'. However, ORMs can also be fitted to a continuous variable as they are intrinsically ordinal in nature (Liu et al. 2017). A significant advantage of this method is that there is no need to convert or reconvert the data during analysis due to the skewness of the substance concentrations, as the distance between ordinal levels is unknown and intrinsically adjusted for within the ORM models. This provides a significant benefit when dealing with chemical substance concentrations in human samples, as extreme outliers can be common, which can produce strong bias in many regression models. Usually, either the participants with outliers would need to be removed from the analysis, or alternatively, the concentrations of the substance would need to be transformed and scaled correctly to reach normality, meeting model assumptions, and then retransformed back, which can introduce biases (Duan 1983). Different transformation and scaling methods can produce differences in results and also potentially reduce the readability of the results (Duan 1983). Additionally, the model is appropriate to use with substances that could not be measured <LOD (Liu et al. 2017), e.g., PFAS values that were converted to a set value (0.0001).

3.4.4 Bayesian kernel machine regression

Bayesian kernel machine regression (BKMR) is a statistical method specifically developed to quantify the health effects of complex chemical mixtures. It has some major advantages over traditional regression methods, particularly in being able to model potential non-linear and non-additive effects while also handling high-dimensionality problems due to large mixtures. Additionally, BKMR was developed with hierarchal selection to account for high correlations between components within a mixture. Due to variable selection methods incorporated within the model, it is possible to identify subsets of the mixture component that are more likely to be included in the mixture exposure effect modelling (Bobb et al. 2015).

3.4.5 Quantile g-computation

Quantile g-computation (QGC) is a method that was developed as an extension of weight quantile sums (WQS) (Keil et al. 2020). WQS was developed to estimate the relative contribution of components within a mixture to estimated health outcomes. WQS converts the exposure to a categorical variable consisting of quantiles, then models a regression between the health outcome and an index of the total mixture exposure whilst adjusting for covariates. Additionally, a set of weights are created for the index exposure. The weights are estimated across various bootstrap samples of the WQS and forced to sum to 1. However, due to optimisation constraints placed on the weights of WQS, directional homogeneity must be assumed, which might not reflect the actual real-world relation of the mixture components and the outcome variable (Keil et al. 2020). QGC, however, can redefine the unidirectional weights as both negative and positive weights, which represent the negative and positive partial effects due to a specific exposure component, effectively circumventing the required assumption of univariate additive effects among the exposures for WQS (Keil et al. 2020).

3.4.6 Ethical approval

Ethical approval for RMA was received from the Regional Ethical Review Board in Uppsala (No 2015/190). Participants from the 8th and 11th grades gave written informed consent to participate, whereas 5th graders had their legal guardians give consent.

4. Results & Discussion

In the following subsections, we will overview the most important results of the three papers included in this thesis. As we examined the same RMA study population across the three papers, we were able to view different analytical angles of the chemical mixture to provide a more holistic understanding of the exposure and effects of chemical mixtures on an adolescent population. Due to the study design of the population recruitment, the results can be generalised to school-attending adolescents across Sweden (Methods, **Paper I**). However, careful consideration should be taken when comparing these results to those of other adolescent studies from countries similar to Sweden due to potential differences in the study populations, statistical models, and covariate adjustments.

4.1 Chemical mixture body burdens

In our first paper, as a part of hazard identification and exposure assessment in the risk assessment procedure, we determined adolescent body burdens of elements and chlorinated/brominated/fluorinated POPs with moderate to long half-lives (months-years) in the body and the rapidly metabolised and excreted substances (hours-days) such as phthalates/phthalate alternatives and bisphenols. We compared RMA body burdens to those found in other adolescent studies conducted around the same time period as RMA (Table 2, **Paper I**). In **Paper I**, we argued that the comparisons between the different studies should not be considered one-to-one as there are subtle but impactful differences in the range of age of the studied populations, sample collection, and presentation of the results. Nevertheless, generally speaking, RMA had similar element concentration levels in whole blood as adolescents from the US (NHANES) (NHANES 2022) and the Flemish region of Belgium

(FLEHS IV) (Schoeters et al. 2022). As for concentration levels of OCPs and PCBs in serum, geometric means were generally slightly lower in RMA than in the US and Germany (GerES) (GerES 2021), but similar to concentration levels reported for the Belgian population. Interestingly, concentration levels of PFAS were also similar to those reported from all of the other three countries (Table 2, **Paper I**), as were concentration levels of most of the substances measured in urine (e.g., phenols, phthalate metabolites) (Table 3, **Paper I**). Considering that all of the adolescent studies in the comparison were from high-income industrialised nations in the northern hemisphere, it is unsurprising that the body burdens were similar since individual chemicals and products containing chemicals are traded more or less freely between these countries. PBDEs were the most prominent exception, with the US reporting higher mean concentrations (NHANES 2022), most probably due to legislation differences regarding flame retardant treatment of consumer products between the US and the other countries (Horton et al. 2013). When we examined possible differences in body burdens based on the participants/maternal birth country income per capita levels in the main ORMs in **Paper II**, we identified that there were large fold-differences between the lowest birth country income adolescent groups compared to the highest birth country income group for certain substances, such as p,p'-DDE and β-HCH (Fig. 2, **Paper II**). This suggests that body burdens of certain compounds among the adolescents at the time of sampling in Sweden are likely to be influenced by various factors associated with a birth country's income per capita level, such as differences in regulatory frameworks, product use, production of chemicals, exposure routes, and general cultural behaviours. As a result, this highlights the difficulty of generalising body burdens of chemical mixtures globally.

4.2 Determinants of body burdens

In **Paper II**, we followed up the exposure assessment of Swedish adolescents by investigating socio-demographic differences in body burdens, including age, gender, birth country, parental education, and latitude/longitude of home address, using ORMs. This enabled us to identify specific groups of adolescents with higher exposure risks than other adolescent groups in Sweden. To understand the relative importance of these main determinants of body burdens in the ORMs, the chi-square of each main determinant was

extracted per model and presented graphically (Fig. S.2, **Paper II**). The cells of the figures are equal to the proportion of the total chi-square for a given model, with the darker blue shading representing a large share of the total chi-square. At a glance, this figure allows us to see which main determinants were more relatively important to the model for each single substance. Comparing it to the figure showing significance levels of the determinants (Fig. 2. **Paper II**), those with higher proportional chi-squares were also generally statistically significant determinants of body burdens.

For the elements, age was the strongest determinant of Se, gender of Mn, Co and Pb, participant/maternal birth country per capita income level of Cd, and latitude of home address of Cr and Ni (Fig. 2 and S.2, **Paper II**). This large variation in important determinants for the elements suggests apparent differences in exposure sources of the elements. Additional covariates were included in secondary ORMs, previously reported as significant determinants of element body burdens in other Swedish populations (Table S.1, **Paper II**). The rationale behind the inclusion of these secondary covariates was to see if the associations with the socio-demographic determinants would remain statistically significant after the inclusion of the additional covariates. Tables S.2 – S.167 for **Paper II** consist of the raw ANOVA and summary results for all main and additional ORMs included in the analysis. However, these tables are not included in the appendix of the thesis and can instead be found on the official journal webpage for the paper (Pineda et al. 2024). None of the significance levels of the most important socio-demographic determinants of the elements were changed markedly by the inclusions of additional covariates (Fig. S.7-S.13, **Paper II**), suggesting that other factors than these additional covariates were behind the associations. The only exceptions were changes in significance levels of less strong determinants such as Cd with gender, Pb with latitude and Al with age (Fig. S.7, S.8, S.12, **Paper II**)

As for the persistent and lipid-soluble POPs, i.e. organochloride pesticides, PCBs, BDE-153, and the water-soluble PFAS; participant/maternal birth country income per capita was generally the most important and significant determinant (Fig. 2 and S.2, **Paper II**). For most substances, the body burdens were, on average, clearly highest among adolescents born in high-income countries by mothers born in high-income countries, which in this study population predominantly meant those born in Sweden (Table 1, **Thesis**; Fig. 3, **Paper II**). Exceptions to this observation

included β-HCH and p,p'-DDE, for which the opposite was apparent (Fig. 3, **Paper II**). The diverging associations for β-HCH and p,p'-DDE were probably due to higher historical and more recent use of DDT and HCH in low-income countries with warmer climates compared to more industrialised high-income countries with cooler climates (Berg et al. 2017). The additional ORM covariates (Table S.1, **Paper II**) did not change the significance levels of the birth country associations (Fig. S.9, **Paper II**).

4.2.1 Birth Country

We suspected that participant/maternal birth country income per capita would be an essential determinant for many of the long half-life lipid soluble POPs that we measured, as early life in-utero exposure and maternal transfer in breastmilk are strong determinants of body burdens for many of these substances (Dekoning & Karmaus 2000; Cottrell et al. 2023; Marchese et al. 2024). We identified a maternal effect in where participants born in high-income countries to mothers born in a lower-income country had notable concentration differences of these substances compared to high-income country participants with mothers also born in high-income countries (Fig. 3, **Paper II**). Yet we were surprised to see that birth country was also a strong determinant for some of the elements that do not transfer readily through the placenta and in breast milk (Parr et al. 1991), and substances measured in urine with very short half-lives in the body (Fig. 2 and S.2, **Paper II**) (Wang et al. 2019). For these substances, there are likely exposure sources, such as household environment, food consumption and product use (Husøy et al. 2019), and other behaviours that are being incorporated within the birth country determinant. However, it is impossible to determine from our data how much the maternal or household factors explicitly contribute to the birth country differences in body burdens. Furthermore, a notable limitation of the birth country variable is that no data was available for time spent in the birth country prior to arrival in Sweden. This meant that we were unable to control for time spent in lower-income countries and, therefore, potential exposure time, especially to the substances with long half-lives. As for the elements and the POPs, the inclusion of additional covariates in the ORMs for the substances measured in urine did not markedly affect the significance levels of the birth country associations, except for one of the DiNP metabolites in urine (Fig. S.9, **Paper II**).

4.2.2 Parental education

Notably, maternal and paternal education levels were not important determinants for many of the studied substances (Fig. 2 and S.2, **Paper II**). We theorise that the participant/maternal birth country income per capita determinant is likely capturing most of the variance incorporated within the maternal and paternal education variables among the Swedish adolescents as maternal and paternal education is typically a proxy for the socio-economic status and habits of a household (Akhlaghipour & Assari 2020). Parental education levels have previously been reported to be significantly associated with child and adolescent body burdens of bisphenols, phthalates, benzophenones, PAHs, PFAS and 3-Phenoxybenzoic acid (3-PBA) (Govarts et al. 2023). However, it should be noted that the EU-wide biomonitoring study made no adjustments for birth country income (Govarts et al. 2023).

4.2.3 Age

Age was a significant and strong determinant for some of the substances analysed in RMA (Fig. 2 & S.2, **Paper II**). To avoid multicollinearity in our models, we did not include school grade as there was a very high correlation between age and school grade (spearman $\rho = 0.94$), and we believe age has more substantial confounding potential in **Papers II & III** than school grade. Notably, a strong positive age association was observed for Se. This increase with age has previously been observed, likely due to increased exposure to Se via food (Wang et al. 1998). Other substances with strong and significant age associations were some DEHP metabolites, 4,4-BPF, and BP-3 (Fig. 2 & S.2, **Paper II**). A strong positive association was also seen for Cd (Fig. S.14, **Paper II**), with the major exposure routes of food and smoking likely to increase with age (osman et al. 2019). A significant negative association was observed with Al and Mn (Fig. S.14, **Paper II**). Interestingly, the significant associations for Cd, Al, and PCBs became insignificant when the additional determinants were included in the ORMs (Table S.1 & Fig. S.7, **Paper II**). The metabolites of DEHP (a plasticiser) had a strong, significant negative association with age, which maps with the evidence that dust inhalation and mouthing are large contributors to children's DEHP exposure, which likely decreases with age (Ginsberg et al. 2016). The additional ORM determinants did not markedly change the DEHP metabolites' significance levels (Table S.1 & Fig S.7, **Paper II**). Furthermore, 4,4-BPF, but not BPA, had a significant and strong positive association with age, possibly due to

exposure sources, including food and receipts (Rochester & Bolden 2015). The antibacterial TCS and the ultraviolet filter BP-3 also had a significant and strong positive association with age, likely due to increased use of cosmetics/hygiene products/ skin products (Weatherly & Gosse 2017; Suh et al. 2020). RMA did not provide data on these potential exposure sources, and thus, they could not be controlled for.

4.2.4 Gender

Gender was another determinant with significant and strong associations with some of the substances analysed in **Paper II**, particularly Mn, Co, Pb, DBP, one PAH metabolite (1-HP), TCS, BHA and BP-3 (Fig. 2 & Fig. S.2, **Paper II**). Generally, we saw that the male participants had higher concentrations than female participants for the POPs and some elements (i.e., Cr, Hg, Pb) but lower concentrations for the substances measured in urine (Fig. S.4, **Paper II**). For POPs and the elements, we hypothesised that the difference may have been due to adolescent males generally consuming more food on average compared to females, as food is a major exposure source for the elements and POPs (Shomaker et al. 2010; Livsmedelsverket 2017). The most substantial fold difference was identified for the food preservative BHA and the ultraviolet filter BP-3 (Fig. S.4, **Paper II**). It is difficult to ascertain why BHA concentrations were higher in female participants than males, as food is a major exposure source for BHA (Zhang et al. 2023a). However, BHA and BP-3 are known to be used as preservatives in cosmetics/hygiene products. We suggested in the paper that the variation may be due to the difference in the uses of cosmetic products between the genders (Dhanirama et al. 2012; Harley et al. 2016; Wnuk et al. 2022). However, for Cd, which showed higher concentrations in females than males, including the additional covariates ‘smoking’ and ‘ferritin levels’ in the ORM, made the gender association non-significant (Table S.1 & Fig. S.8, **Paper II**). Smoking is a significant source of Cd exposure (Bárány et al. 2002). Low iron status causes increased body burdens of Cd due to increased intestinal Cd uptake by iron uptake mechanisms (Bárány et al. 2005). Female adolescents are much more prone to have iron deficiency than males due to menstruation bleeding (Domellöf & Sjöberg 2024), which could at least partially explain the non-significant gender association after including the additional covariates. Additionally, menstruation in female adolescents may also have contributed to the lower PFAS levels in females since menstrual bleeding is

a known elimination route of PFAS (Upson et al. 2022). In RMA, females who had started menstruating tended to have lower body burdens of some PFASs than those who reported not having started menstruating (Nyström et al. 2022b).

4.2.5 Latitude & longitude

Latitude and longitude of home address were identified as important determinants for a few substances based on significance (Fig. 2, **Paper II**) and the proportion of chi-square (Fig. S.2, **Paper II**). Details of the estimated adjusted concentrations following the latitudinal line north and longitudinal line east were generated for significantly associated substances (Figs. 4 & S.19, **Paper II**). Amongst the elements, latitude was the most crucial determinant for Cr and Ni. When inspecting the estimated adjusted concentrations for Cr, we see an increase towards 60°N before the confidence intervals inflated, making the prediction unreliable further north (Fig. 4, **Paper II**). Eastwards we saw an increase till 15°E and a sharp decrease thereafter (Fig. S.19, **Paper II**). Cr generally tends to be ubiquitous across the environment. However, it is known that the energy industry and urban environments can increase exposure, which might influence the results (Tumolo et al. 2020). For Ni, there is a slight decreasing concentration trend the more north a participant lived and almost no change eastwards (Figs. 4 & S.19, **Paper II**). Ni is ubiquitous across different environments, so it is unclear why concentrations are higher in participants living in the south of Sweden compared to the north (Genchi et al. 2020). Food is most likely an important exposure source to both elements (Livsmedelsverket 2017), and some of the differences could be due to regional variations in diet.

Latitude was an important and significant determinant for BDE-47 and -99 (Fig. 2 & S.2, **Paper II**); when reviewing the estimated adjusted mean concentrations, we see a clear and steady increase for BDE-47 throughout the latitudinal range and a sharp increase for BDE-99 up to ~58°N before plateauing and having the confidence intervals inflate making the prediction less reliable further north (Figs. 4, **Paper II**). We suspect the increase in flame-retardant concentrations further north may be due to differences in housing conditions, as dust ingestion is a major exposure factor for brominated flame retardants (Malliari & Kalantzi 2017).

4.3 Vaccine antibody titres

There has been a growing body of evidence suggesting that chemical exposure may influence antibody titres in children and adolescents, with a specific focus on PFAS (Zhang et al. 2022; Crawford et al. 2023). However, studies on other chemical substances have been sparse, with virtually no studies examining associations with large, complex chemical mixtures. Therefore, as part of the hazard characterisation of the substance mixture studied in RMA, we investigated the effects of chemical mixtures consisting of elements and POPs against DTP and MR vaccine antibodies in **Paper III**, using a sub-section of the population examined in **Papers I & II** (Fig. 2, **Thesis**). Due to the single-spot urine sampling, we decided against including the substances measured in urine in the BKMR models. Our rationale is that due to the very short half-lives of the substances, exposures measured at the time of sampling would likely not represent long-term exposure. Taken together with the understanding that immune development is a long-term process (Simon et al. 2015), any associations, if found, between acute exposure at the time of sampling and antibody titres would therefore be highly uncertain.

In the BKMR modelling, the estimated posterior inclusion probabilities (PIP) results provided us with an overview of which substances in the mixture were important for each antibody model, based on the inclusion probability of the second half of all the models Markov chain Monte Carlo (MCMC) iterations (Section 3.2 & Table 1, **Paper III**). As explained in the paper, most substances had relatively low PIPs, with only the pertussis model having group PIPs ≥ 0.33, which we classified as the cut-off for a substance being 'moderately important' to the model. It should be noted that these classifications are simply arbitrary, and there is no consensus in the literature for classifications or cut-off points. Care must be taken not to conflate PIPs with 'statistical significance', a frequentist concept that is irrelevant to Bayesian statistical methods. As was noticed later in the results, PIPs are not necessarily reflective of a substance's effects on the antibody outcome; for example, in the diphtheria model, BDE-153 showed a relatively strong positive univariate exposure-effect but had a gPIP of 0.015 (Fig. 1 & Table 2, **Paper III**).

Our results showed that the overall chemical mixture we examined in the RMA study population had generally weak and limited effects on all the examined antibody titres (Fig. 2, **Paper III**). The limited effect changes also

extended to the univariate analysis, where we studied the individual effect of each substance in the mixture on antibody titres (Fig.1, **Paper III**).

Reviewing the literature, we were only able to find one other study that examined an adolescent population using a national cohort, NHANES, which had some of the same exposures (i.e., PFAS) and some of the same antibody titres included in our paper (MR), which also utilised BKMR (Zhang et al. 2023b). Interestingly, the study found a distinct negative association between the PFAS mixture and rubella antibody concentrations. This contrasted our results, which revealed a slight positive but low confidence association of the whole joint mixture effect, which included but was not limited to PFAS (Fig. 2, **Paper III**). In our univariate analysis, PFAS generally had no effect and otherwise had more positive trends than negative trends in the DTP and MR models (Fig. 1, **Paper III**). We reasoned and demonstrated that generally, the examined RMA study population had severalfold lower PFAS concentrations in comparison to the NHANES studies, which may be contributing to the differences in results (Discussion, **Paper III**). It should be noted that the Zhang et al. study did find a positive overall trend between the PFAS mixture and measles antibodies, but with credible intervals overlapping the null, which we also found in our more complex mixture analysis (Fig. 2, **Paper III**) (Zhang et al. 2023b).

Based on the BKMR results, there is evidence to suggest that there is a mixture effect on antibody titres in RMA, but the effect is relatively low and hard to detect in an epidemiological setting. A systematic review of PFAS associations with various vaccine antibodies also found similar evidence, reporting consistent evidence of immunosuppression but with overall log coefficients being close to null (Crawford et al. 2023). The same review also found statistically significant effect modification based on early life-stage (childhood) exposure for PFOA and PFOS (Crawford et al. 2023).

4.4 Risk characterisation

In **Paper I**, we examined the basic body burdens of the RMA participants and compared the results to HBM-GVs obtained from various published sources (Table 4, **Paper I**) in an effort to perform a risk characterisation of the chemical mixture measured in RMA. We identified various substances examined in the studied RMA population, which had a significant portion of the RMA population exceeding HBM-GVs. Pb had 12% of participants (17% male/ 8.8% female) exceeding the 1% benchmark dose level (BMDL1%) of developmental neurotoxicity in children (Table 4, **Paper I**). Additionally, Pb had a 7.1% (8.4% male/6.1% female) exceedance of the BDML1% for systolic blood pressure. BDML is the dose correlated with an increased percentage risk of a particular health outcome in a given population. In **Paper II**, we identified that Pb concentrations are higher in the north of Sweden compared to the south (Fig. 4, **Paper II**). It is unclear as to why there is an increase in Pb concentrations in the north of Sweden, but due to the high total percentage exceedance of the guidance values, further investigation is required. A possible exposure hypothesis includes hunting, as most ammunition is typically made from Pb, with elevated Pb body burdens identified in Swedish adults that consume wild game (Wennberg et al. 2017). Interestingly, the latitude association became non-significant when the additional covariates were added to the Pb ORM (Fig. S.12, **Paper II**), which included game consumption but also smoking, alcohol and seafood consumption and season of sampling (Table S.1, **Paper II**).

There were no published HBM-GV for Al at the time of writing **Paper I**. Instead, we opted to use an occupation threshold for adverse neuropsychological effects in adults, such as fatigue, depression and memory/concentration problems (Riihimäki et al. 2000). An alarming 26% (27% male/25% female) of the studied RMA population exceeded this threshold. Podalgoda et al. published a HBM-GV at 8 µg/L for adults in plasma or serum for Al based on the tolerable weekly intake (TWI) of Al established by EFSA in 2008 (EFSA 2008; Poddalgoda et al. 2021), which is slightly higher than the occupational threshold suggested by Riihimäki et al. (2000). The new Al HBM-GV, based on the EFSA TWI, included neurotoxicity as a critical endpoint in the risk assessment (EFSA 2008; Poddalgoda et al. 2021). Using this newly proposed HBM-GV resulted in 18% of the RMA participants exceeding the value, a still relatively high percentage. **Paper II** identified age, participant/maternal birth country, and

longitude as significant socio-demographic determinants for Al. In **Paper I**, there were no marked school grade differences in the percentage exceeding the occupational threshold (Table 4, **Paper I**). Food is very likely to be a major exposure source for Al, with cereal products and sugar/sweets contributing the highest average intake levels (Livsmedelsverket 2017). However, over-the-counter drugs, mainly antacids, may contain high levels of Al, and even short-term use (i.e., weeks) of some of these drugs may drastically increase the body burdens of Al (Gräske et al. 2000). No information about the use of Al-containing drugs was available in RMA. Further investigation is urgently required to determine exposure sources and if other residual confounding determinants may explain the exposure of Swedish adolescents to Al, as regulatory measures may be required to limit the exposure.

When combining PFOA, PFNA, PFHxS and PFOS, ($PFAS_4$) serum concentrations, 21% of participants had concentrations exceeding the serum level that corresponds to the recommended TWI for young women, based on immunotoxicity in toddlers being exposed from the mother in utero and through breastfeeding (EFSA 2020). Additionally, PFOA had 12% (12% male/13% female), and PFOS had 19% (27% male/14% female) of the participants above HBM I guidance values established by the German Human Biomonitoring Commission (Hölzer et al. 2021). The HBM I values for PFOA and PFOS were based on mixed toxicity, including immunotoxicity, lower birth weight alterations in lipid metabolism and hormone disruption (Nyström et al. 2022b), signifying a threshold for early warning and concern of exposure. The most significant associated determinants identified for PFOA and PFOS were gender and participant/maternal birth country income per capita level (Fig. 2 & S.2, **Paper II**). In **Paper I**, there was no difference in the percentage of exceedances of the PFOA HBM I value between males and females, but for PFOS, males exceeded notably more than females (Table 4, **Paper I**). Additionally, the differences in body burdens between males and females were higher for PFOS than for PFOA (Fig. S.4, **Paper II**). Furthermore, 5^{th} graders in RMA had a larger proportion of exceedances of the PFOS HBM I value than participants from grades 8 and 11 (Table 4, **Paper I**). This was mainly due to the inclusion of one school in southern Sweden (i.e., Ronneby) (Fig. 1, **Paper II**), with a history of high PFOS contamination in their drinking water (Xu et al. 2021). Lower concentrations of PFOA and PFOS

were associated with participants and mothers born in lower-income countries compared to when both were born in high-income countries (Fig. 2, **Paper II**), which, in this case, predominantly meant compared to those born in Sweden (Table 1, **Thesis**). Hypothetically, exposure sources related to birth country income, such as food and drinking water consumption, product use, and in-utero and breastmilk exposure, may contribute to adolescents exceeding the HBM-GVs. Drinking water and fish/shellfish consumption were previously highlighted as the main dietary exposure determinants of PFAS within RMA (Nyström et al. 2022a; Nyström-Kandola et al. 2023).

4.4.1 Hazard index

Three target organs were used for the HI analysis, which was determined based on literature specifying the critical effect behind published HBM-GVs or HBGV (Table 4, **Paper I**). These three target organs were the liver, the kidneys and the CNS. Two substances contributed to the liver HI, four to the kidney HI and six to the CNS target organ (Table 5, **Paper I**). HIs > 1 signifies participants with combined HRs of different substances on the same target organ that could be regarded as a health concern of combined exposure. The HI results can be found in Table 5 of **Paper I**, but briefly, the liver (3.2% with HI > 1) and kidney (1.5%) had relatively low HI exceedances, but the CNS (94%) had an alarming result. Median HI for the CNS was 1.82, with a maximum of 15. This indicates that the joint HR exceedance was not magnitudes over the HBM-GVs for about half of the participants. Although this is a concerning result, it should be noted that exceedance of HI cannot be directly equated with a risk of disease occurrence. Additionally, CNS HI has an inherent limitation in that it is based on a broad assumption that all of the six substances included (Hg, Pb, Al, non-dioxin-like PCBs, BDE-99, 3-PBA) have an additive effect on the CNS with the same mode of action. This is unlikely to be the case, as the CNS is a complex system with various domains and mechanisms, where potentially, each substance may interact with different sections (Vuong et al. 2020). Still, further investigations are required to see if there is, in fact, an additive or interactive mixture effect occurring on the CNS within the Swedish adolescent population.

5. Strengths & Limitations

As discussed in all three papers, a significant advantage of this thesis is the RMA study population. As Statistics Sweden designed the school classroom invitations to participate to be representative of the whole school-attending demographic of Sweden, and 99% of adolescents are enrolled in secondary school (WBG 2020), we can generalise the results across the entire Swedish adolescent population. This provides us with a high degree of confidence that the results are accurate and representative of the Swedish adolescent population and greater confidence in the analysis generated.

Another notable strength of the thesis is the inclusion of an exposure assessment on a large and complex chemical mixture. Other population studies in the literature typically have focused on single substances or substance groups instead. RMA provides a more holistic overview of the exposure situation for Swedish adolescents and the socio-demographic differences in the patterns of exposure (Figures, **Papers II**). This holistic overview of chemical mixture exposure was also brought into the BKMR modelling of associations with vaccination antibodies (Figures, **Paper III**). Additionally, due to including a complex chemical mixture in the BKMR models, we were able to identify interactions within the models between substances from vastly different substance groups, including elements, OCPs, and PBDEs.

Antibody titres are generally considered a reasonable endpoint for assessing immunotoxicity via immunosuppression and subsequent risk of infection, making it useful for risk assessment (Luebke et al. 2004; Crawford et al. 2023). Additionally, a previous systematic review found significantly fewer mixture studies focusing on immunotoxic endpoints compared to others (Martin et al. 2021). However, as we argued in **Paper III**, it is unclear if single-spot sampling of vaccine antibodies accurately represents the

participants individual vaccine response. Wide variability exists in participants 'baseline' antibody titres and may not directly reflect the overall immune health of a participant, as responses to different vaccines are not correlated (Zimmermann et al. 2021).

The immune system is very complex and influences on antibody titres may only represent the effects on specific parts of the immune system. Protective thresholds for antibody titres are also not currently established for pertussis or MR. Although thresholds have been established for diphtheria, they have been based on the deaths/development of neurological complications caused by diphtheria in seven Swedish individuals having <0.01 IU/mL diphtheria toxoid antibodies (WHO 2009b). Yet there is no precisely well-defined protective limit due to variation in protection, and therefore, full protection is instead assumed to be >0.1 IU/mL (Ölander et al. 2009; WHO 2009b; Zasada et al. 2013). We proposed a different study design for future adolescent studies, previously used in children (Grandjean et al. 2012; Timmermann et al. 2020), where pre- and post-vaccine sampling is performed to provide a vaccine response difference, which may better represent the immunotoxic effects of the mixture, if present. This would also alleviate the limitation of cross-sectional studies of adolescents of different ages due to large differences in 'time since last vaccination', as decay rates of diphtheria and tetanus antibodies are relatively rapid per year compared to other vaccines (Antia et al. 2018), with some of the RMA participants having up to 12 years since the last DTP vaccination (Table 1, **Paper III**).

Epidemiological studies are vital for detecting signals in a sea of data and 'background noise' and can provide a 'blueprint' for further investigation. However, alone, they are insufficient in explaining the complex interactions, associations, causal effects, and mechanisms underpinning the relationship between an observed health effect and substance or mixture exposure. An obvious example that can be taken from this thesis is the interactions we identified in **Paper III**. In the tetanus model, we identified that BDE-47 interacts with BDE-153 by increasing the positive exposure-response effect of BDE-153 when BDE-47 is set at its 10^{th} percentile concentration compared to its median and 90^{th} percentile (Fig. S.3, **Paper III**). We could argue that based on the data, it appears that BDE-47 has an antagonistic interaction with BDE-153. However, in the same model analysis, PeCB interacts with p,p'-DDE by decreasing the positive effect at lower p,p'-DDE concentrations when PeCB concentrations are set at the median and 90^{th}

percentile compared to the 10th percentile (Fig. S.3, **Paper III**); it is much more challenging to classify if this interaction is antagonistic or synergistic. Due to this ambiguity, we avoided classifying the interactions within **Paper III**. Furthermore, from this analysis alone, it is impossible to determine if observed interactions are generalisable to other population groups, if the relation is genuinely antagonistic or synergistic, and what underlying molecular mechanisms underpin this relationship. This highlights the general limitations of relying only on epidemiological studies and why it is essential to incorporate a multi-discipline approach to risk-assessing chemical mixtures. With further experimental in-vitro, in-vivo and epidemiological studies, we are more likely to determine if there are casual relationships and understand the fundamental molecular mechanisms underpinning the relationship.

An issue with the BKMR analysis is the communication of the results; as discussed earlier, the Bayesian statistical framework does not generally employ 'statistical significance' and instead provides posterior probabilities based on a defined credible interval. There are strong arguments against the use of 'statistical significance' due to its many limitations (Wasserstein et al. 2019). However, an advantage of its use is the ability to quickly convey, specify and separate the 'impactful and important' results from the 'noise'. Additionally, BKMR results need to be reviewed in a holistic manner as each analysis (i.e., overall joint effects, PIPs, univariate, single-variable, bi-variate) is an equally valid interpretation of the same high-dimensional model (Bobb et al. 2018). Focusing on only one or some of the analyses can easily lead to misinterpretation of the results. Yet, because of the variety of analyses available in BKMR, in conjunction with a complex and extensive mixture, it can also be challenging to represent the results cohesively.

For **Paper II**, we opted to use MICE to impute missing data at random values to try and keep as many of the study participants in the statistical modelling as possible. As for **Paper III**, we decided against using MICE as there was no readily available implementation to incorporate the multiple iterations directly into the BKMR methodology. This was, however, a limitation as we lost some participants and, therefore, statistical power due to missing values in their data.

For **Papers I, II & III**, we had several substance values <LOD. We chose to use the machine reads rather than impute <LOD, although this may create more uncertainty for the substances with high percentage of values <LOD.

This was a particularly important decision for **Paper III**, as a study comparing statistical methods for analysing interactions found that imputing for values <LOD led to the loss of interactive effects within the mixture (Kundu et al. 2024). We attempted to mitigate the uncertainty by only including substances that had <30% of total values <LOD in the final analyses (Methods, **Papers II & III**).

6. Conclusions & Future Perspectives

The studies included in this thesis attempt to illuminate the complex and multifaceted relationship between chemical mixtures and human health, with a particular focus on adolescent exposure in Sweden. By analysing various chemical groups, including those with long and short half-lives, we have underscored the pervasive nature of chemical exposure in modern life, the body burdens and its potential implications for health and well-being. The varied roles and potential toxicities of essential and non-essential elements, persistent organic pollutants, phenols, phthalates and other toxic substances have been examined, highlighting the importance of understanding individual and combined exposures and effects.

Paper I identified the body burdens of the RMA population and highlighted several substances that may pose a health concern to the population at current levels. In particular, Pb, Al, and PFAS4 were highlighted for having a high percentage (i.e., ≥10%) of the studied Swedish adolescent population exceeding HBM-GVs. Furthermore, the HI analysis suggests a possible CNS health risk based on the very conservative assumption that there are additive mixture effects of Hg, Pb, Al, non-dioxin-like PCBs, PBDE-99, and 3-PBA on the CNS. More in-depth investigations of combined neurotoxicity are needed to determine if the assumptions are valid and, if so, to investigate associations, exposure sources and underlying health effects and mechanisms.

Paper II examined the association of socio-demographic determinants with the examined chemical mixture and, in particular, highlighted birth country per capita income levels for both the participants and mothers as crucial variables for epidemiological studies of chemical mixtures. The birth country of both the participant and the mother may, in many cases, be related to both the mixture exposure and the health outcomes studied, thus being

potentially important confounders. Many studies have highlighted the importance of parental education, especially maternal education, as a determinant of exposure levels to toxic substances. In RMU, parental education levels were fairly weakly related to mixture exposure, and we speculate that the birth country income variable, to a large extent, explains the education level associations reported by others. Additionally, the RMA study found many significant associations of substances to latitude and longitude of home address in Sweden, and the results highlighted that, apart from age and gender disparities in exposures, there are geographical inequalities in exposure to various of the examined substances. It is hoped that future comprehensive studies will delve into identifying the sources and behaviours leading to socio-demographic disparities in adolescent exposure to chemical mixtures, as well as potential health outcomes, both within Sweden and globally. The objective should be to minimise these inequalities in chemical exposure to the greatest extent possible.

Paper III explored the potential association of the chemical mixture exposure with vaccine antibody titres of DTP and MR. Although some positive and negative trends were found for individual substances and the mixture as a whole, the effect change remained relatively small. However, the study also found interesting evidence suggesting various substances, such as BDE-47 and PeCB, may be interacting with other substances in the mixture. Further investigations are warranted, particularly with revised study designs that take into account early-life exposure.

All three papers highlighted the inherent complexities and difficulties of examining large, complex mixtures within an epidemiological context. Research into chemical mixtures is still an emerging field. The research outlined in this thesis emphasises the critical need for continued rigorous investigation into the toxicity of chemical mixtures. It points to the importance of developing more sophisticated models for predicting mixture effects, considering the interactions and combined impacts of chemical exposures. Advances in purpose-built statistical methods such as BKMR allow researchers to try and identify the non-linear associations in the overall mixture effect and the individual substance effect while also allowing for the examination of potential interactions. However, there is still no ‘gold-standard’ set, with substantial need and room for even better tools that can further reduce the dimensionality of mixture models to allow for better estimates of outcomes and easier conveyance of the results. Advances in

several machine-learning methods show promise; however, they are potentially limited due to ambiguity as to how model variables influence the results, as is the case in neural networks, or due to a requirement of high participant numbers for model training and testing (Zhu et al. 2024). Yet, new tools are being developed to explain machine learning weights, and some initiatives are being taken on at an EU-wide level to collect and standardise more exposure data (Luijten et al. 2023; Zhu et al. 2024).

By fostering a deeper understanding of the complex mixture exposure and interactions between chemical mixtures and human health, this thesis contributes to advancing the field of toxicology and risk assessment. Some of the findings advocate for more research and potentially stronger regulatory frameworks to limit human exposure to potentially harmful chemicals, especially among vulnerable populations such as adolescents. Ultimately, it underscores the necessity of balancing the benefits of chemical use in society with the imperative to protect human health and the environment, guiding future research and policy decisions in a direction that ensures a safer and healthier world for generations to come.

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Popular science summary

The Unseen Hazards in Our Daily Lives: The Intricate World of Chemical Mixtures

In our modern world, chemicals are everywhere – from the clothes we wear and the food we eat to the very air we breathe. Chemicals are indispensable in driving innovation forward and supporting the continued sustainable industrialisation of our societies. With global chemical production and use continuously increasing, our chemical reliance is only expected to grow. Yet, amidst this background of rapid technological advancement, a critical question looms large: what are the implications of our increasing exposure to chemical mixtures?

The complexity of the chemical mixtures we are exposed to from our environment, which may contain a large number of different chemicals, presents a formidable challenge in toxicology. These mixtures don't simply combine the individual traits of the single chemicals; they may also interact in unpredictable ways. This complexity is not just an academic puzzle – it may have profound implications for our health.

This thesis focuses on a particular population group usually underrepresented in the scientific literature, adolescents. Recognising this, using the Riksmaten adolescents 2016-17 study population, consisting of roughly 1000 participants with ages between 10 and 21 years, the thesis attempts to identify the current body burdens of various toxic substances in the Swedish adolescent population, the socio-demographic factors associated with such body burdens, and the potential immunological health impacts of these substance mixtures.

Particular attention is paid to very diverse groups of chemicals – some persisting in our bodies for years, like certain toxic metals and persistent organic pollutants, and others that are gone within hours or days from our

bodies but are omnipresent in an adolescent's environment, including plastics, cosmetics and personal care products.

Major findings of this thesis include that a participant's birth country, but also their mother's birth county, is a major contributor to the chemical body burden profile that adolescents will likely have. We also identified differences in exposure based on gender and geographically where the participant homes were located. We identified various substances with concerning levels above recommended health-based guideline values in parts of the adolescent population, particularly regarding lead, aluminium, and per- and poly-fluoroalkyl substances and (PFAS). Our results also suggest that the examined chemical mixture may pose a health concern to the central nervous system (CNS), but this assessment is based on the assumption that the chemicals act in the same way on the CNS, which is not likely. Further investigations are needed in this matter. Additionally, we observed that these studied chemical mixtures affected the levels of vaccination antibodies to a limited extent, but this study only provides an initial insight into potential chemical mixture effects on the complex immune system in adolescents.

This thesis revealed disparities in mixture exposure among Swedish adolescents born in different countries, as well as gender and regional exposure differences. Addressing these inequalities is essential to achieving the United Nations' sustainability goals of equality within and between countries and gender. The RMU study serves as a crucial reminder of the delicate chemical balance society must maintain. Understanding the interactions and potential effects of chemical mixtures is not just an academic endeavour but a vital step toward safeguarding public health. As we continue to advance technologically towards a sustainable society, this thesis points toward the need for vigilant oversight and continued research into the environmental and health impacts of our chemical-dependent lifestyle.

Populärvetenskaplig sammanfattning

De osynliga farorna i vårt dagliga liv: den invecklade världen av kemiska blandningar

I vår moderna värld finns kemikalier överallt – från kläderna vi bär och maten vi äter till själva luften vi andas. De är oumbärliga för att driva innovation framåt och stödja den fortsatta utvecklingen mot ett hållbart samhälle. Som ett resultat av den globala utvecklingen förväntas vårt beroende av kemikalier bara att öka. Ändå, mitt i denna snabba tekniska utveckling, dyker en kritisk fråga upp: vad är konsekvenserna av vår ökande exponering för kemiska blandningar? Komplexiteten hos kemiska blandningar utgör en stor utmaning inom toxikologin. Kemikalierna kombinerar inte bara utgående från sina individuella egenskaper; de kan i vissa fall interagera på ibland oförutsägbara sätt. Denna komplexitet är inte bara ett akademiskt pussel – den kan också ha oväntade konsekvenser för vår hälsa, beroende på de exponeringsnivåer som vi utsätts för. Avhandlingen är ett första steg i undersökningar av de kemikalieblandningar som ungdomar i Sverige exponeras för och de potentiella hälsorisker som dessa blandningar kan medföra. Ungdomar som deltog i den riksomfattande kostundersökningen Riksmaten Ungdom 2016-17 (RMU) lämnade blod- och urinprov för analys av olika kemikalier, som ett mått på den exponering de utsatts för. Avhandlingen ägnar särskild uppmärksamhet åt några olika grupper av kemikalier – som i vissa fall finns kvar i våra kroppar i åratal, till exempel potentiellt toxiska metaller och organiska klorerade/bromerade/fluorerade föroreningar (POPar), men även andra kemikalier som försvinner ur kroppen inom några timmar/dagar men som är kontinuerligt närvarande i ungdomarnas miljö, till exempel i vardagsprodukter som plast, kosmetika, hudvårdsprodukter och hygienprodukter. Viktiga resultat i denna avhandling är att ungdomarnas

exponeringsmönster för kemikalierna liknar de som observerats i andra högt industrialiserade länder såsom Tyskland, Belgien och USA, med undantag av POParna polybromerade difenyletrar (PBDE) som förekommer i mycket högre halter bland ungdomar från USA. RMU visade också att ungdomarnas exponeringsmönster för kemikalierna varierade, bland annat beroende på kön, var de bor, och var både de och deras mammor är födda. Ungdomarnas födelseland var en särskilt viktig faktor för exponeringsmönstren, där ungdomar födda i högt industrialiserade länder, främst Sverige, hade klart högre halter av POParna polyklorerade bifenyler (PCB), PBDE och per- och polyfluorerade alkylsubstanser (PFAS) än ungdomar födda i utvecklingsländer innan de flyttade till Sverige. Ett undantag bland POParna var de klorerade bekämpningsmedlen DDT och β-hexaklorcyklohexan, vilka förekom i klart högre halter hos ungdomar födda i utvecklingsländer. Dessa ungdomar hade också högre halter av de snabbt utsöndrade kemikalierna som finns i många olika vardagsprodukter än ungdomar födda i Sverige.

En första riskbedömning av ungdomarnas exponering antyder att kemikalier som kan påverka det centrala nervsystemet bör noggrant studeras vidare för mer säkra slutsatser om dessa kemikalier är ett hälsoproblem. I en första studie av kemikaliernas potentiella effekter på ungdomarna immunförsvar undersöktes samband mellan exponering och mängden vaccinationsantikroppar i blodet efter vaccinering mot difteri, stelkramp och kikhosta (DTP), samt mässling och röda hund (MR). Svaga och osäkra samband mellan ökad exponering för kemikalierna och lägre mängder DTP-antikroppar observerades, medan sambanden för MR var det motsatta. Dessa svaga samband kan till exempel bero på att vissa av substanserna motverkar varandras effekter eller att de exponeringsnivåer som ungdomarna utsatts för inte var tillräckligt höga för att ge starka samband.

Denna avhandling identifierar viktiga skillnader i exponeringsmönstret för de studerade kemikalierna bland svenska ungdomar födda i olika länder, men också exponeringsskillnader beroende på kön och var i landet ungdomarna bor. Dessa ojämlikheter i exponering måste åtgärdas för att vi ska kunna nå FN:s hållbarhetsmål om jämställdhet inom och mellan länder, och jämställdhet mellan könen. Blandningsexponeringarna verkade inte vara tillräckligt höga för att markant påverka antikroppsnivåer mot DTP och MR, men denna avhandling är bara en första undersökning av möjliga kemiska blandningseffekter på det komplexa immunsystemet hos ungdomar.

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Appendix

I. Exposure of Swedish adolescents to elements, persistent organic pollutants (POPs), and rapidly excreted substances – The Riksmaten adolescents 2016-17 national survey

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Exposure of Swedish adolescents to elements, persistent organic pollutants (POPs), and rapidly excreted substances – The Riksmaten adolescents 2016-17 national survey

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ABSTRACT

Adolescence is a period of significant physiological changes, and likely a sensitive window to chemical exposure. Few nation-wide population-based studies of chemical body burdens in adolescents have been published. In the national dietary survey Riksmaten Adolescents (RMA) 2016–17, over 13 chemical substance groups, including elements, chlorinated/brominated/fluorinated persistent organic pollutants (POPs) were analysed in blood, and in urine metabolites of phthalates/phthalate alternatives, phosphorous flame retardants, polycyclic aromatic hydrocarbons (PAHs), and pesticides, along with bisphenols and biocide/preservative/antioxidant/UV filter substances (N = 1082, ages 11–21). The aim was to characterize the body burdens in a representative population of adolescents in Sweden, and to compare results with human biomonitoring guidance values (HBM-GVs). Cluster analyses and Spearman's rank order correlations suggested that concentrations of substances with known common exposure sources and similar toxicokinetics formed obvious clusters and showed moderate to very strong correlations ($r \geq 0.4$). No clusters were formed between substances from different matrices. Geometric mean (GM) concentrations of the substances were generally less than 3-fold different from those observed among adolescents in NHANES (USA 2015–16) and GerES V (Germany 2014–17). Notable exceptions were brominated diphenyl ethers (PBDEs) with >20-fold lower GM concentrations, and the biocide triclosan and ultraviolet (UV) filter benzophenone-3 with >15-fold lower mean concentrations in RMA compared to NHANES. Exceedance of the most conservative HBM-GVs were observed for aluminium (Al, 26% of subjects), perfluorooctanesulfonic acid (PFOS, 19%), perfluorooctanoic acid (PFOA, 12%), lead (Pb, 12%), MBP (dibutyl phthalate metabolite, 4.8%), hexachlorobenzene (HCB, 3.1%) and 3-phenoxybenzoic acid (PBA, pyrethroid metabolite, 2.2%). Males showed a higher proportion of exceedances than females for Pb, HCB and PFOS; otherwise no gender-related differences in exceedances were observed. A higher proportion of males than females had a Hazard Index (HI) of substances with liver and kidney toxicity and neurotoxicity >1. Industrialized countries with similarly high standards of living, with some exceptions, show comparable average body burdens of a variety of toxic chemicals among adolescents from the general population. The exceedances of HBM-GVs and HIs strongly suggests that further efforts to limit chemical exposure are warranted.

1. Introduction

Chemicals are used in almost every aspect of society and are found ubiquitously in human exposure media, including food, drinking water, air, dust, and consumer products. There are currently over 26,000 unique substances registered with the European Chemicals Agency, of which approximately 1000 are considered potential endocrine

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Abbreviations

Al	aluminium
BBOEP	bis(2-butoxyethyl) phosphate
BBzP	butylbenzyl phthalate
BHA	3-tert-butyl-4-hydroxyanisole
BMDL1%	benchmark dose of 1% risk
BPS	bisphenol S
BPA	bisphenol A
BP3	benzophenone-3
Cd	cadmium
Co	cobalt
Cr	chromium
br-PFOS	branched perfluorooctanesulfonic acid
CNS	central nervous system
cx-MiDP	mono-carboxy-isononyl phthalate
cx-MiNP	mono-(4-methyl-7-carboxyheptyl) phthalate
cx-MINCH	cyclohexane-1,2-dicarboxylate-mono (7-carboxylate-4-methyl)heptylester
DBP	dibutyl phosphate
p,p'-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
p,p'- DDT	dichlorodiphenyltrichloroethane
DEP	diethyl phthalate
DEHP	diethylhexyl phthalate
DiDP	diiso-decyl phthalate
DiNCH	diisononyl-cyclohexane-1,2-dicarboxylate
DiNP	diiso-nonyl phthalate
DPHP	dipropylheptyl phthalate
EFSA	The European Food Safety Authority
GC-MS/MS	gas chromatography – triple quadrupole mass spectrometry
GM	geometric mean
HBM-GVs	human biomonitoring guidance values
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
Hg	mercury
HI	Hazard Index
HR	Hazard Ratio
ICP-MS	inductively coupled plasma mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
L-PFOS	linear perfluorooctanesulfonic acid
L-PFHxS	linear perfluorohexanesulfonic acid
L-PFOA	linear perfluorooctanoic acid
MEHP	mono(2-ethylhexyl) phthalate
MEP	monoethyl phthalate
Mn	manganese
MBP	monobutyl phthalate
Ni	nickel
OCPs	organochlorine pesticides/metabolites
OH-MiNP	mono-(4-methyl-7-hydroxyoctyl) phthalate
OH-MINCH	cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester
OH-MPHP	6-hydroxy monopropylheptyl phthalate
oxo-MiNP	mono-(4-methyl-7-oxooctyl) phthalate
PAHs	polycyclic aromatic hydrocarbons
PBDEs	polybrominated diphenyl ethers
PCBs	polychlorinated biphenyls
Pb	lead
PFDA	perfluorodecanoic acid
PFNA	perfluorononanoic acid
PFUnDA	perfluoroundecanoic acid
PFAS	per- and polyfluoroalkyl substances
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
POPs	persistent organic pollutants
RMA	Riksmaten Adolescents
Se	selenium
SFA	Swedish Food Agency
TPP	triphenyl phosphate
TBEP	tri(2-butoxyethyl) phosphate
TCS	triclosan
TCP	trichloropyridinol
UBA	German Environment Agency
UPLC	ultra performance liquid chromatograph
US	United States
UV	ultraviolet
1-HP	1-hydroxy-pyrene
2-OH-PH	2-hydroxy-phenanthrene
3-PBA	3-phenoxybenzoic acid
4,4-BPF	bisphenol F
5-cx-MEPP	mono-(2-ethyl-5-carboxypentyl) phthalate
5-OH-MEHP	mono-(2-ethyl-5-hydroxyhexyl) phthalate
5-oxo-MEHP	mono-(2-ethyl-5-oxohexyl) phthalate

disrupting compounds (ECHA, 2022; Street et al., 2018). Furthermore, approximately 1000 new chemicals are registered with the United States Environmental Protection Agency per year (EPA, 2022), often with unknown or poorly understood safety profiles. Many chemicals are known to interfere with the human hormonal system and have previously been associated with a wide range of negative health consequences including neurologic, reproductive and other physiological effects (Braun, 2017; Wan et al., 2021; Yilmaz et al., 2020). Thus, considering both the number and effects of chemicals to which humans are exposed, understanding the variation of chemical mixture exposures within human populations (i.e., the chemical 'exposome') is of great importance.

Most studies examining human exposure to chemicals focus on single compound groups, such as elements, per- and polyfluoroalkyl substances (PFAS) and polychlorinated biphenyls (PCBs) (Marques et al., 2021; Preston et al., 2020; Roth et al., 2021), with few looking at the wider part of the chemical exposome. In reality, human exposure to chemicals is a complex process which involves multiple substances and pathways. Combination toxicity may occur when multiple chemicals occurring at sufficiently high concentrations act on a common endpoint to elicit an effect (Kortenkamp, 2014). In general, such mixtures may elicit a combination effect by either 'concentration addition' or 'independent action', although synergistic or antagonistic effects are also possible, albeit less frequently observed in experimental studies (Martin et al., 2021). 'Concentration addition' occurs when a toxicity threshold is exceeded by the sum concentration of multiple chemicals with the same mechanism/mode of action. In comparison, 'independent action' may occur when chemicals with different modes-of-action reach their individual toxicity thresholds on the same endpoint, thus eliciting a toxic effect (Kortenkamp, 2014). Concentration addition presents a unique challenge to risk assessors because individual toxic compounds may not be found in sufficiently high concentrations in study populations to pass observable health-risk thresholds, but may exceed such thresholds in combination with other chemicals sharing the same mechanism/mode of action. Further layers of complexity arise whereby chemical substances with independent modes of action can still share the same adverse health effects (Christiansen et al., 2020).

Biomonitoring of chemical body burdens in blood and urine provides valuable information about cumulative exposure at the time of sampling. Adolescence is likely a sensitive developmental window to

chemical exposure due to the significant physiological changes that occur. Nevertheless, few nation-wide studies have been published on adolescent exposure to a broad range of chemicals. The few well-known examples include NHANES in the United States (US) and GerES V in Germany (GerES, 2021; NHANES, 2022). Additionally, FLEHS IV in Belgium (Schoeters et al., 2022) represents another similar (albeit regional) study on the chemical exposome of adolescents. These studies, to the best of our knowledge, have not fully investigated the correlations between exposure of adolescents to the measured substances/substance groups. Knowledge of such correlations, which may be due to common exposure sources and/or similarities in toxicokinetics, are crucial when interpreting associations between health outcomes and body burdens of chemical mixtures. The previous studies have compared the observed concentrations of measured chemicals with human biomonitoring guidance values (HBM-GVs), in order to identify the chemicals with concern for adolescent health. However, they have not investigated possible gender and age differences in this context, which may be important for health development during this period of rapid physiological change. Moreover, to the best of our knowledge, no such study has attempted to investigate possible combination toxicity risks with substances with common target organs.

The overall aim of the present study is to characterize the body burdens of over 60 different substances across 13 chemical substance groups (elements (e.g., heavy metals), chlorinated, brominated, and fluorinated persistent organic pollutants (POPs), phthalates, phthalate alternatives, bisphenols, phosphorous flame retardants, polyaromatic hydrocarbons (PAH), pesticides, biocides/preservatives and UV-filters) in Swedish adolescents. To address this goal, we compared measured concentrations in whole blood, serum and urine to those reported in similar studies from the United States, Germany and the Flanders region of Belgium. We also examined correlations among individual substances, in an attempt to identify shared sources of exposure. Finally, we compared measured concentrations to published HBM-GVs and determined Hazard Indices in an effort to assess cumulative health risk (Kortenkamp and Faust, 2010).

2. Method

2.1. Study group and design

The study population was a sub-sample of Riksmaten Adolescents 2016–17 (RMA), a cross-sectional national population-based study of dietary habits and chemical exposure of Swedish adolescents, conducted by the Swedish Food Agency (SFA). Details about the recruitment process, study design and data collection are described elsewhere (Moraeus et al., 2018). Briefly, 619 schools were invited, being representative of the entire country across the 5th (median age, range: 11 years, 10–13), 8th (14 years, 11–15) and 11th (17 years, 16–21) school grades. Of these schools, 259 were invited to participate in the biomonitoring part, with 62 school classes across 57 unique schools opting-in (Moraeus et al., 2018) (Fig. S1, supplement). In total, 1305 students, out of 2377 invited, accepted to be sampled and 1082 had sufficient biomonitoring data to be included in our analysis (Table 1 & Table S1, Supplement). The study design included online-based questionnaires (RiksmatenFlexQ) addressing background factors such as age and gender (Moraeus et al., 2018).

2.2. Ethics

Ethical approval was obtained from the Regional Ethical Review Board in Uppsala (No 2015/190). Participants in grades 8 and 11 gave written informed consent to participate in the study, whilst legal guardians gave consent for 5th graders.

Table 1
Gender differences in substance concentrations among RMA participants.

Substances[a]		Geometric mean (95% CI)			p-value[b]
Abbreviation	Unit/ Matrix	Male (N = 476)	Female (N = 606)	Total (N = 1082)	
Cr	µg/L whole blood	0.58 (0.57–0.60)	0.55 (0.54–0.57)	0.57 (0.56–0.58)	0.092
Mn	µg/L whole blood	10.1 (9.67–10.4)	11.0 (10.7–11.2)	10.6 (10.3–10.8)	<0.001
Co	µg/L whole blood	0.11 (0.10–0.11)	0.14 (0.14–0.15)	0.13 (0.12–0.13)	<0.001
Ni	µg/L whole blood	0.62 (0.61–0.64)	0.62 (0.61–0.64)	0.62 (0.61–0.63)	0.466
Se	µg/L whole blood	94.7 (91.8–97.7)	95.0 (93.9–96.1)	94.8 (93.4–96.3)	0.141
Cd	µg/L whole blood	0.11 (0.11–0.12)	0.13 (0.13–0.14)	0.12 (0.12–0.13)	<0.001
Hg	µg/L whole blood	0.75 (0.69–0.81)	0.60 (0.56–0.64)	0.66 (0.63–0.69)	<0.001
Pb	µg/L whole blood	8.17 (7.77–8.58)	6.72 (6.49–6.97)	7.32 (7.11–7.55)	<0.001
HCB	pg/mL serum	47.3 (45.8–48.9)	38.5 (37.3–39.8)	42.2 (41.2–43.2)	<0.001
p,p'-DDE	pg/mL serum	124 (115–133)	99.7 (93.2–107)	110 (104–115)	<0.001
PCB-118	pg/mL serum	7.01 (6.67–7.36)	6.41 (6.14–6.70)	6.67 (6.45–6.89)	0.006
PCB-138	pg/mL serum	30.3 (28.6–32.2)	23.3 (22.1–24.6)	26.2 (25.2–27.2)	<0.001
PCB-153	pg/mL serum	49.0 (46.1–52.1)	36.9 (34.9–39.0)	41.8 (40.0–43.6)	<0.001
PCB-170	pg/mL serum	13.7 (12.8–14.7)	9.86 (9.24–10.5)	11.4 (10.9–12.0)	<0.001
PCB-180	pg/mL serum	27.7 (25.7–29.8)	19.7 (18.5–21.1)	22.9 (21.8–24.1)	<0.001
PCB-187	pg/mL serum	6.14 (5.71–6.61)	4.49 (4.20–4.80)	5.15 (4.90–5.41)	<0.001
L-PFOA	ng/g serum	1.26 (1.21–1.31)	1.17 (1.13–1.22)	1.21 (1.18–1.25)	0.001
PFNA	ng/g serum	0.39 (0.37–0.41)	0.34 (0.32–0.35)	0.36 (0.35–0.37)	<0.001
PFDA	ng/g serum	0.15 (0.14–0.16)	0.15 (0.14–0.16)	0.15 (0.14–0.16)	0.219
L-PFHxS	ng/g serum	0.55 (0.50–0.61)	0.39 (0.36–0.42)	0.45 (0.43–0.48)	<0.001
L-PFOS	ng/g serum	2.43 (2.29–2.59)	1.92 (1.82–2.03)	2.13 (2.05–2.22)	<0.001
br-PFOS	ng/g serum	1.12 (1.05–1.19)	0.85 (0.80–0.90)	0.96 (0.92–1.00)	<0.001
MEP	ng/mL urine	31.5 (28.8–34.6)	51.0 (46.4–56.2)	41.3 (38.6–44.2)	<0.001
MBP	ng/mL urine	38.9 (36.5–41.3)	43.7 (41.6–45.9)	41.5 (39.9–43.1)	0.001
MBzP	ng/mL urine	6.33 (5.73–6.99)	7.61 (6.98–8.30)	7.02 (6.57–7.49)	0.006
MEHP	ng/mL urine	1.61 (1.52–1.71)	1.82 (1.72–1.93)	1.73 (1.66–1.80)	0.005
5OH-MEHP	ng/mL urine	7.81 (7.35–8.29)	8.19 (7.69–8.73)	8.02 (7.67–8.38)	0.230
5oxo-MEHP	ng/mL urine	5.98 (5.62–6.37)	6.69 (6.31–7.10)	6.37 (6.10–6.65)	0.033
5cx-MEPP	ng/mL urine	6.80 (6.39–7.24)	7.69 (7.25–8.16)	7.29 (6.98–7.61)	0.010
2cx-MEHP	ng/mL urine	2.05 (1.92–2.18)	2.18 (2.06–2.32)	2.12 (2.03–2.22)	0.120
OH-MiNP	ng/mL urine	4.23 (3.89–4.61)	4.93 (4.52–5.38)	4.61 (4.33–4.91)	0.042
oxo-MiNP	ng/mL urine	1.94 (1.79–2.09)	2.34 (2.16–2.54)	2.15 (2.04–2.28)	0.001

(continued on next page)

Table 1 (*continued*)

Substances[a]		Geometric mean (95% CI)			p-value[b]
Abbreviation	Unit/ Matrix	Male (N = 476)	Female (N = 606)	Total (N = 1082)	
cx-MiNP	ng/mL urine	6.34 (5.84–6.88)	7.89 (7.27–8.57)	7.17 (6.76–7.60)	0.001
cx-MiDP	ng/mL urine	0.36 (0.34–0.39)	0.44 (0.42–0.47)	0.41 (0.39–0.43)	<0.001
OH-MPHP	ng/mL urine	1.07 (1.00–1.15)	1.16 (1.08–1.24)	1.12 (1.07–1.18)	0.189
oxo-MiNCH	ng/mL urine	1.20 (1.07–1.33)	1.36 (1.24–1.50)	1.29 (1.20–1.38)	0.032
cx-MINCH	ng/mL urine	0.88 (0.78–0.98)	1.01 (0.92–1.12)	0.95 (0.88–1.03)	0.034
OH-MINCH	ng/mL urine	0.92 (0.82–1.03)	0.99 (0.89–1.10)	0.96 (0.89–1.03)	0.321
DPP	ng/mL urine	1.82 (1.71–1.93)	2.13 (2.01–2.25)	1.99 (1.91–2.07)	0.001
DBP	ng/mL urine	0.13 (0.12–0.14)	0.17 (0.15–0.18)	0.15 (0.14–0.16)	<0.001
BPA	ng/mL urine	0.87 (0.80–0.95)	0.88 (0.81–0.95)	0.88 (0.83–0.93)	0.536
BPS	ng/mL urine	0.12 (0.11–0.13)	0.16 (0.15–0.18)	0.14 (0.14–0.15)	<0.001
4,4BPF	ng/mL urine	0.09 (0.08–0.11)	0.11 (0.10–0.13)	0.10 (0.09–0.11)	0.026
2-OH-PH	ng/mL urine	0.16 (0.15–0.17)	0.17 (0.16–0.19)	0.17 (0.16–0.18)	0.160
TCP	ng/mL urine	1.24 (1.16–1.31)	1.35 (1.28–1.43)	1.30 (1.25–1.35)	0.021
3-PBA	ng/mL urine	0.27 (0.25–0.29)	0.29 (0.28–0.31)	0.28 (0.27–0.30)	0.029
TCS	ng/mL urine	0.25 (0.23–0.29)	0.33 (0.30–0.37)	0.30 (0.27–0.32)	0.002
BHA	ng/mL urine	0.27 (0.21–0.36)	1.33 (1.05–1.69)	0.66 (0.55–0.80)	<0.001
BP3	ng/mL urine	0.64 (0.56–0.73)	1.56 (1.35–1.81)	1.05 (0.95–1.17)	<0.001

[a] Substances with over 50% of samples < LOQ or LOD are not included in this table. For the purpose of calculating geometric means and 95% confidence intervals (CI), determined concentrations < LOQ or < LOD were used and concentrations determined to be 0 were converted to the lowest concentration above 0 divided by sqrt(2) (see section Handling of data below LOQ/LOD). When LOD was available PFAS concentrations < LOD were converted to LOD/sqrt(2), else concentrations < LOQ were converted to LOQ/sqrt(2).

[b] Wilcoxon rank sum test on comparison of concentrations by gender. p-values <0.05 indicate significant difference of concentration between genders.

2.3. Sample collection

One single-spot non-fasting blood and urine sample, along with anthropometric data, were collected from each participant by trained staff during school visits on weekdays throughout 2016 (September)-2017 (May). Blood samples (10 ml) for serum were collected in Vacutainer serum tubes with coagulation activator (Becton Dickinson, article # 367896, Sweden), centrifuged at 1500×*g* for 10 min and then aliquoted to cryotubes. Whole blood samples (4 ml) were collected in lithium-heparin Vacuette tubes (Greiner Bio-one, article# 454056, Germany). Urine samples were collected in acetone-rinsed paper cups by the individual participants during the school visit, and these samples were then aliquoted to polypropylene tubes. All aforementioned samples were promptly frozen at −20 °C at the site of collection and shipped frozen to the SFA where they were stored at −80 °C until being sent to external laboratories for analysis. None of the samples analysed in this study had known disruptions in the cold chain.

2.4. Sample analysis

Full names and abbreviations of the analysed substances, CAS numbers, and their main uses are provided in Table S2 (Supplement). Decisions on inclusion of substances were made by experts from the SFA at the time of study design and were based on toxic properties of the substances and presence in food and/or drinking water. Some essential/suspected essential elements were also included based on suspected interactions with non-essential toxic elements in the body (chromium (Cr), manganese (Mn), selenium (Se), cobalt (Co)) (Table S2, supplement).

2.5. Elements

Samples were analysed for Cr, Mn, Co, nickel (Ni), Se, cadmium (Cd), mercury (Hg), lead (Pb), and aluminium (Al) by the Department of Laboratory Medicine, Lund University, Lund, Sweden (Table S2, Supplement). Concentrations of Pb, Cd and Hg in RMA have been published previously (Almerud et al., 2021). Sample analyses were performed in duplicate. In short, the samples were treated as previously described (Barany et al., 1997). The concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientific, Bremen, GmbH) equipped with collision cell with kinetic energy discrimination and helium as collision gas. The limit of detection (LOD) varied from 0.05 to 5.0 μg/L. Method precision varied from 2.8 to 15% depending on analyte.

2.6. PCBs, organochlorine pesticides/metabolites (OCPs) and polybrominated diphenyl ethers (PBDEs)

The Department of Health Security, National Institute for Health and Welfare, Kuopio, Finland analysed the chlorinated and brominated POPs in serum (Table S2, Supplement) as described previously (Gasull et al., 2019). Some results from the analyses have been published previously (Zamaratskaia et al., 2022). In brief, concentrations were measured using gas chromatography – triple quadrupole mass spectrometry (GC-MS/MS). The instrument used was an Agilent 7010 GC-MS/MS system (Wilmington, DE, U.S.), GC column DB5MS UI (J&W Scientific, 20m, ID 0.18 mm, 0.18 μm). Limits of quantification (LOQ) ranged from 5 pg/mL for PCB congeners and trans-nonachlor to 40 pg/mL for 1, 1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE). Two blank samples and two control samples (NIST SRM 1958) were included in each batch of samples. Measured concentrations of chlorinated and brominated POPs in SRM1958 were 80–105% of the certified/reference concentrations. The coefficient of variation (CV%) from SRM 1958 (n = 18) was <3.6% for all compounds.

2.7. PFAS

Serum samples were analysed for PFASs by the Department of Environmental Science, Stockholm University, Sweden (Table S2, Supplement). Details of the extraction and instrumental analysis are described in Nyström et al. (2022). Briefly, samples were fortified with a suite of isotopically-labelled internal standards and then extracted twice with acetonitrile in an ultrasonic bath, followed by clean-up with acidified graphitized carbon. The cleaned-up extract was diluted and fortified with volumetric standards and thereafter stored at −20 °C prior to analysis. Instrumental analysis was carried out on a Acquity ultra performance liquid chromatograph coupled to a Waters Xevo TQS triple quadrupole mass spectrometer operated in negative electrospray ionisation, multiple reaction monitoring mode. Quantification was based on isotope dilution or an internal standard approach (see Nyström et al. (2022) for details). LOQs are summarized in Table S3 (Supplement). In the present study only data on selected perfluoroalkyl acids: linear perfluorooctanoic acid (L-PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), linear perfluorohexanesulfonic acid (L-PFHxS), branched and linear perfluorooctanesulfonic acid (br-PFOS, L-PFOS), were used due to the low detection frequency of other PFASs analysed in RMA (Nyström et al., 2022).

2.8. Substances measured in urine

Analyses were performed at the Department of Laboratory Medicine, Lund University, Sweden, as previously described (Alhamdow et al., 2017; Cequier et al., 2014; Liljedahl et al., 2021) with some modifications. In brief, the samples were analysed on a liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS; QTRAP 5500, AB Sciex, Framingham, MA, USA). Two urinary quality control samples were included in the analysis, one authentic and one spiked. The CV% calculated from QC samples included in all sample batches (N = 34) as a between-run precision, did not exceed 20% for almost all compounds with the exception of mono-carboxy-isononyl phthalate (cx-MiDP, 27% at concentration of 0.6 ng/mL), bisphenol S (BPS, 25% at concentration of 0.8 ng/mL), dibutyl phosphate (DBP, 41% at concentration of 0.1 ng/mL) and 3-tert-butyl-4-hydroxyanisole (BHA, 21% at concentration of 0.8 ng/mL). The analyses of bisphenol A (BPA), 1-hydroxy-pyrene (1-HP), benzophenone-3 (BP3), triclosan (TCS), 3-phenoxybezoic acid (3-PBA), trichloropyridinol (TCP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5-oxo--MEHP), is part of G-EQUAS inter-laboratory control program (University of Erlangen-Nuremberg, Germany). The laboratory also participates in the HBM4EU QA/QC program, and has qualified as HBM4EU laboratory for the analysis of: BPA, BPS, BPF, 1-HP, monobenzyl phthalate (MBzP), mono-(2-ethylhexyl) phthalate (MEHP), 5-OH-MEHP, 5-oxo--MEHP, mono-(2-ethyl-5-carboxypentyl) phthalate (5-cx-MEPP), mono-(4-methyl-7-hydroxyoctyl) phthalate (OH-MiNP), mono-(4-methyl-7-oxooctyl) phthalate (oxo-MiNP), mono-(4-methyl-7-carboxyheptyl) phthalate (cx-MiNP), cyclohexane-1,2-dicarboxylate-mono (7-carboxylate-4-methyl)heptylester (cx-MINCH), cyclohexane-1,2-dicarboxy late-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH). Urine concentrations were adjusted for individual differences in urine density in the RMA population as described by (Carnerup et al., 2006). LODs are summarized in Table S4 (Supplement).

2.9. Handling of data below LOQ/LOD

The number of samples with concentrations below LOQ/LOD for each substance are presented in Tables S3 and S4 (Supplement). Except for PFASs, concentrations below LOQ or LOD were handled as previously described (Darnerud et al., 2015; Gyllenhammar et al., 2017; Svensson et al., 2021). Briefly, concentrations above 'background noise' in the analyses were determined to the concentration estimated in the analytical run. When a concentration was estimated not to be above 'background noise' in the chemical analyses, the concentration was set to zero (AMC, 2001). In the case of PFAS, concentrations below LOQ but above LOD were used when available, while concentrations below LOD were set to zero. Concentrations determined to be below LOD or LOQ are more uncertain and should be regarded as semi-quantitative. Nevertheless, the use of these determined concentrations in statistical analyses, as opposed to using substituted or imputed data, is generally considered to result in less statistical bias (AMC, 2001; Bergstrand and Karlsson, 2009). Fig. S2 (Supplement) provides examples of Al, PCB-183 and bis(2-butoxyethyl) phosphate (BBOEP) showing the change in concentration distribution of the substances after replacement of concentrations < LOQ or LOD with determined concentrations instead of fixed concentrations at LOQ or LOD/$\sqrt{2}$. Nevertheless, it is important to be aware of that use of data with a large proportion of determined concentrations below LOQ or LOD will add uncertainty in the interpretation of the results. For GM calculations, concentrations determined to be zero were by necessity further converted to the lowest determined concentrations above zero divided by $\sqrt{2}$. In these cases, PFAS concentrations below LOQ or LOD were set to LOQ or LOD/$\sqrt{2}$.

2.10. Statistical analysis

Analysis was performed using *R* (ver: 4.0.2). In the cluster analysis, the 'varclus' function from the 'hmisc' package (ver: 4.4–0) was used to visualise the clustering of the chemical substances using spearman correlation coefficients with complete linkage. Correlation analyses of relationships between substance concentrations were assessed using Spearman Rank Correlation coefficients. By using results from the cluster analysis in combination with the correlation analysis it is possible to get a more comprehensive description of the relationships between individual substances. It is usually not probable that the cluster analysis identifies clusters including all substances that are similarly correlated to one another. In this case, the correlation analysis provides information about correlations between substances separated in different clusters. In both the cluster and the correlation analysis, substances with more than 70% of concentrations above zero were included in the analyses (Tables S3 and S4, supplement), with the full range of values used for calculations. The strength of the correlations was assessed using definitions proposed by (Schober et al., 2018), where a correlation coefficient of 0.00–0.39 is classified as negligible to weak, 0.40–0.69 is moderate, 0.70–0.89 is strong, and 0.90–1.00 is a very strong correlation. Wilcoxon rank sum and signed rank test ($\alpha = 0.05$) was used to analyse if there were gender-related differences in concentrations, and a Kruskal-Wallis rank sum test ($\alpha = 0.05$) to analyse if there are significant concentration differences in at least one of the school grades compared to the others. The Chi-Squared Test was used to test if there were gender- or school grade-related differences in proportions of exceedances of HBM-GVs and HI > 1 (see section 'Comparisons with HBM-GVs and HI assessment' below).

2.11. Study comparison

The literature was searched for nation-wide population-based studies that analysed the larger chemical exposome in both blood and urine amongst adolescents during the same period as RMA. We found two studies matching this criteria, United States NHANES 2015–17 (NHANES, 2022), German GerEs V 2014–17 (GerES, 2021), and additionally the regional Flemish Belgium FLEHS IV 2016–2020 (FLEHS, 2020).

Concentrations for NHANES were mostly reported as weighted GMs. In the case of chlorinated and brominated POPs, concentrations were instead reported as arithmetic means of pooled samples from separate ethnic groups and genders. We therefore included the reported minimum and maximum ethnical group/gender mean concentrations reported in NHANES 2015-17 within our comparison table (Table 2). Values for GerES V were reported as GMs but sometimes included younger children than those included in RMA in the reporting of certain substances. Values for FLEHS IV were reported as GMs for the adolescent study population, ages 14–15. Comparatively, we reported the RMA results as GMs including all ages (ages 10–21) analysed within our study population.

2.12. Comparisons with HBM-GVs and HI assessment

To assess the potential health concerns associated with chemical exposure in Swedish adolescents, HBM-GVs were obtained for the investigated substances from the peer-reviewed literature. We focussed on published values from the German Environment Agency (UBA) and the HBM4EU project (Apel et al., 2020; UBA, 2015) (Table 4). If HBM-GVs were published by both organizations, the HBM4EU value was used. In certain cases when HBM-GVs from these two sources were lacking we used published biomonitoring equivalents (Hays and Aylward, 2009), or published health-based values from other sources that could be used when comparing with our observed concentrations (Table 4). While the search for HBM-GVs was not exhaustive, data were obtained for many of the substances in the present work.

In order to examine possible combination toxicity of measured substances, the HI assessment approach was used (Borg et al., 2013). This approach assumes concentration addition between measured substances

Table 2
Comparison of geometric mean (GM) of substance concentrations in whole blood and serum observed in RMA with those reported for 12–19 years old adolescents from NHANES, USA, 3–17 years old children/adolescents from GerES V, Germany, and 14–15 years old adolescents from the Flemish regions of Belgium (FLEHS IV)[a].

Substances[b]	RMA (2016–2017) GM (N = 1082)	NHANES (2015–2016) Weighted GM[c] (N = 353–565)	GerES (2014–2017) GM (age) (N = 516–2256)		FLEHS IV (2016–2020) GM (N = 428)
Whole blood (μg/L)					
Mn	11	11			9.4
Se	95	190			
Cd	0.12	0.13	<0.12	(12–17)	0.19
Hg	0.66	0.40			
Pb	7.3	4.7	8.4	(12–17)	7.7
Serum (pg/ml)[c]					
HCB	42	24–37000 (NHBF – AHM)	<70	(14–17)	25
beta-HCH	3.1	<4–60 (NHBF - AHM)	<20	(3–17)	3.7
Oxychlordane	0.13	<4–7900 (NHBF - AHM)			4.0
trans-Nonachlor	2.9	9.3–12000 (NHBF – AHM)			2.5
p,p'-DDT	0.16	<4–16000 (NHBF - AHM)	<20	(14–15)	5.9
p,p'-DDE	110	160. – 690000 (NHBF - AHM)	134	(14–17)	135
PCB-74	3.5	<2–51 (NHWF - NHWM)			
PCB-99	3.9	0.2–3800 (MAF - AHM)			
PCB-118	6.7	4.1–5500 (MAF - AHM)	<20	(3–17)	
PCB-138	26	6.2–10000 (MAF - AHM)	45	(14–17)	
PCB-153	42	8.2–13000 (MAF - AHM)	62	(14–17)	
PCB-156	3.8	<0.8–6.2 (NHBF - NHWM)			
PCB-170	11	2.1–2900 (NHBF - AHM)			
PCB-180	23	4.1–7000 (MAF - AHM)	32	(14–17)	
PCB-183	2.1	<0.8–4.1 (NHBF - AM)			
PCB-187	5.2	2.1–2300 (MAF - AHM)			
∑PCB-138, 153, 180	92		140		71
BDE-47	0.78	27–83 (AF - NHBM)			<1
BDE-99	0.29	7.9–20 (AF - MAM)			<1
BDE-153	0.68	16–54 (AF - NHBM)			<1
Serum (ng/g serum)[d]					
L-PFOA	1.2	1.1	1.1	(12–17)	1
PFNA	0.36	0.47	<0.49	(3–17)	0.3
PFUnDA	0.10	<0.10	<0.24	(3–17)	
PFHxS	0.45	0.89	0.35	(12–17)	0.47
L-PFOS[f]	2.1	2	2.4	(12–17)	2.1
br-PFOS	0.96	0.85			

[a] (GerES, 2021; NHANES, 2022; Schoeters et al., 2022).
[b] For the purpose of calculating geometric means in RMA, determined concentrations < LOQ or < LOD were used and concentrations determined to be 0 were converted to the lowest concentration above 0 divided by sqrt(2) (see section Handling of data below LOQ/LOD). When LOD was available, PFAS concentrations < LOD were converted to LOD/sqrt(2), else concentrations < LOQ were converted to LOQ/sqrt(2).
[c] NHANES/GerES/FLEHS; ng/g lipid of chlorinated and brominated POPs converted to pg/ml serum using average lipid content of serum in RMA of 0.33%.
[d] NHANES/GerES/FLEHS; PFAS ng/mL converted to ng/g using the specific gravity of serum (1.0275) (Sunderman and Boerner, 1949).
[e] Chlorinated POPs; arithmetic mean in pooled samples (N = 2–11, each consisting of 8 individuals) from different ethnical groups: NHBF = non-hispanic black females. AHM = all hispanic males. MHWF = non-hispanic white female. NHWM = non-hispanic white male. MAF = mexican american females. AM = asian male. AF=Asian female. MAM = Mexican american male.
[f] GerES & FLEHS linear + branched isomers.

in the assessment of possible cumulative health concerns. A conservative approach was used by grouping substances with the same target organ of critical effects (Table 4), specifically in this present study kidney toxicity, liver toxicity and neurotoxicity. Each participant's Hazard Ratio (HR) of the substance in question was calculated by dividing the measured concentration of the substance and its most conservative HBM-GV. Each individual's HRs were summed together to a HI according to the shared target organ of critical effects (see formula below). A potential concern to human health exists if the HI is > 1 for each target organ. See Table 5 for selected substance HRs included within each HI.

$$\text{Hazard Index} = \frac{C1}{GV1} + \frac{C2}{GV2} + \frac{C3}{GV3} + \ldots\ldots\ldots\frac{Ci}{GVi}$$

C_i: concentration of substance i in serum/whole blood/urine. GV_i: the HBM-GV for substance i (see Table 4). Hazard Index = the sum of the HRs of each substance in an individual with the same target organ of critical effects (see Table 5)

3. Results and discussion

RMA showed ubiquitous exposure of Swedish adolescents to mixtures of many of the studied elements, chlorinated pesticides, PCBs, and PFASs (Table 1 & Table S3, Supplement). Moreover, >50% of the participants had quantifiable concentrations of urinary metabolites of phthalates/phthalate alternatives, as well as metabolites of phosphorous flame retardants, PAHs, and pesticides, and bisphenols, and biocide/preservative/UV filter chemicals (Table 1 and Table S4, Supplement). This is in line with results from similar studies on adolescents from Germany and the USA (GerES, 2021; NHANES, 2022), and the regional study in Belgium (Schoeters et al., 2022).

Many of the studied elements and POPs have half-lives in the human body ranging from months to years (Nordberg et al., 2014; Verner et al., 2009); consequently, concentrations are representative of medium-to long-term cumulative exposure prior to sampling. In contrast, urine substances have short elimination half-lives (i.e., hours to a few days) and are therefore representative of very recent exposure (Perrier et al., 2016). Nevertheless, the almost ubiquitous quantification of these 'short-lived' substances in adolescent urine suggests that exposure

Table 3
Comparison of geometric means (GM) of substance concentrations in urine observed in RMA, with those reported for 12–19 years old adolescents from NHANES and 14–17 years old adolescents from GerES.

Substances	RMA (2016–2017) GM (N = 1082)	NHANES (2015–2016)[a,b] Weighted GM (N = 405)	GerES (2014–2017)[c] GM (N = 516–2256)	FLEHS IV (2016–2020)[d] GM (N = 416)
Urine (ng/mL)				
MEP	41	35	26	38
MBP	42	12	21	20
MBzP	7	6.1	3.1	3
MEHP	1.7	1.2	1.4	1.1[e]
5OH-MEHP	8	5.8	11	6.7
5oxo-MEHP	6.4	3.8	7.6	4.2
5cx-MEPP	7.3	9.4	12	16
OH-MiNP	4.6		6.9	3.9[e]
oxo-MiNP	2.2	2.6	2.8	
cx-MiNP	7.2		5.9	1.7[e]
cx-MiDP	0.41	2.2	0.9	1.2[e]
OH-MPHP	1.1		0.3	
oxo-MiNCH	1.3		0.93	
cx-MINCH	0.95	0.58	1.1	0.98[e]
OH-MINCH	0.96	0.97	2.3	1.2[e]
DPP	2	1.4		
DBP	0.15	0.21		
BPA	0.88	1.2	1.9	1.1
BPS	0.14	0.37		0.13
4,4BPF	0.10	<0.2		0.17
2-OH-PH	0.17	0.06	0.09	
1-HP	0.07	0.16	0.12	
3-PBA	0.28	0.64		
TCS	0.30	5.2	<1	
BP3	1.1	16	<2	

[a] DPP and DBP from 2013 to 2014 instead. 2-OH-PH from 2011 to 2012 instead. 1-HP and 3-PBA from 2013 to 2014 instead.
[b] (NHANES, 2022) Volumes II & III, updated March 2021.
[c] (Murawski et al., 2020; Schwedler et al., 2020a, 2020b; Tschersich et al., 2021).
[d] (Schoeters et al., 2022).
[e] (Bastiaensen et al., 2021).

occurred on a more-or-less daily basis in Sweden during the RMA study period. However, the use of a single-spot urine sample from each participant makes it difficult to estimate individual long-term exposure due to large intra-individual temporal variation in exposure (Perrier et al., 2016). Nonetheless, the RMA results give estimates of the overall exposure situation among adolescents in Sweden 2016–17 (Aylward et al., 2017).

3.1. Correlations between substance concentrations

This is, to the best of our knowledge, one of the most comprehensive studies of relationships between whole blood, serum and urine concentrations of chemicals in a national population study of adolescents. In the cluster analysis, most elements with diverging dietary exposure sources (Livsmedelsverket, 2017), did not form clear cluster groupings and in most cases negligible to weak correlations were observed (Fig. 1 and Fig. S3, Supplement). Moreover, with a few exceptions, correlations between elements and POPs were negligible to weak (Fig. S3, Supplement), most likely due to differences in toxicokinetics (much shorter half-lives of most elements, many POPs being lipid soluble) (Andersen et al., 2021; Nordberg et al., 2022; Verner et al., 2009) and/or exposure sources (mainly plants-based foods vs foods of animal origin) (Livsmedelsverket, 2017). Within-group clusters were observed among the POPs with similar dietary exposure patterns (Krauskopf et al., 2017; Lignell et al., 2011; Livsmedelsverket, 2017; Nyström et al., 2022) and toxicokinetics (Andersen et al., 2021; Verner et al., 2009) (Fig. 1). However, no overlaps were observed between the chlorinated, brominated and fluorinated POPs (Fig. 1). As suspected from the very short half-lives and diverging exposure sources, the urine substances did not cluster together with any of the substances measured in blood (Fig. 1) and correlations were negligible to weak (results not shown). Among substances measured in urine, metabolites of diethylhexyl phthalate (DEHP), diiso-nonyl phthalate (DiNP), and diisononyl-cyclohexane-1, 2-dicarboxylate (DiNCH) clustered separately from one another and displayed moderate to strong correlations within a given cluster (Fig. 1 & Fig. S4, Supplement). Se and Hg, and Co and Cd, formed separate clusters among the elements (Fig. 1). Hypothetically, Se and Hg relationships could be due interactions between these elements in the body (Bárány et al., 2003; Bates et al., 2006; Glynn and Lind, 1995; Ralston and Raymond, 2010). For Co and Cd similar dietary exposure sources (cereals and sugar/sweets) (Livsmedelsverket, 2017) and toxicokinetics (sharing of iron-transporting mechanisms) (Bárány et al., 2005) may have contributed. Hexa-to hepta-chlorinated PCBs clustered separately from tetra-to penta-chlorinated PCBs as well as from the chlorinated pesticides (Fig. 1). The indoor environment may still be a significant exposure source of the latter PCBs (Johansson et al., 2003; Meyer et al., 2013; Wingfors et al., 2006). For HCB, hexachlorocyclohexane (HCH) and p,p'-DDE, fish consumption has been a less important dietary source than for PCBs in Sweden (Livsmedelsverket, 2017; Törnkvist et al., 2011). BDE-47 and -99 formed a cluster separate from BDE-153 (Fig. 1), whereas BDE-153 was moderately correlated with highly chlorinated PCBs (Fig. S3, Supplement), as also observed among nursing women from Sweden (Lignell et al., 2011). The indoor environment appears to be a more significant source of human BDE-47 and BDE-99 exposure than of BDE-153 exposure (Björklund et al., 2012; Fromme et al., 2016). Among young Swedish women, BDE-153 body burdens were significantly related to consumption of fish (as was the case for certain PCBs), whereas no such relationship was found for BDE-47 and -99 (Lignell et al., 2011). As in a previous RMA study using PCA clustering (Nyström et al., 2022), L-PFHxS, L-PFOS and br-PFOS clustered separately from L-PFOA, PFNA, and PFDA (Fig. 1), which was suggested to be due to exposure to the former PFASs from drinking water (Nyström et al., 2022).

Apart from phthalates/phthalate alternatives with common 'mother

Table 4
Estimated fraction (%) of exceedances of toxic substance concentrations among RMA participants (N = 1082) in relation to non-cancer HBM-GVs, gender and school grade differences in exceedances, and critical effects of the HBM-GVs.

Substance (metabolite)	Type of HBM-GV[a]	Matrix	HBM-GV	Participants exceeding HBM-GV (%)	Male/female participants exceeding HBM-GV (%)	Participants in grade 5/8/11 exceeding HBM-GV (%)	Critical effect	Reference for HBM-GV
Se	Biomonitoring equivalents (BE)	Whole blood	400 μg/L	0			Selenosis	Hays et al. (2014)
Cd	BE	Whole blood	1.4 μg/L	0.8			Kidney toxicity	Hays et al. (2008)
Hg	Human biomonitoring I (HBM I) children/adults	Whole Blood	5 μg/L	0.4			Neurotoxicity	Apel et al. (2017)
	HBM II children/adults	Whole blood	15 μg/L	0			Neurotoxicity	Apel et al. (2017)
Pb	BMDL1% developmental neurotoxicity children	Whole blood	12 μg/L	12	17/8.8*	12/13/11	Neurotoxicity	EFSA (2010)
	BMDL1% systolic blood pressure	Whole blood	15 μg/L	7.1	8.4/6.1	7.3/7.4/6.6	Hypertension	EFSA (2010)
	BMDL10% chronic kidney disease	Whole blood	36 μg/L	0.4			Kidney toxicity	EFSA (2010)
Al	Occupational threshold adverse effects	Serum	6.8 μg/L	26	27/25	27/26/25	Neurotoxicity	Riihimäki et al. (2000)
HCB[b]	BE	Serum	80 pg/mL	3.1	5.9/1.2*	2.7/4.4/2.3	Liver toxicity	Aylward et al. (2010)
p,p'-DDE[b]	Safe level developmental effects	Serum	12.8 ng/mL	0			Reproductive toxicity	WHO (2012)
NDL-PCB[c]	HBM I (138 + 153+180x2)	Serum	3.5 ng/mL	0			Neuro-/immunotoxicity	Apel et al. (2017)
	HBM II	Serum	7 ng/mL	0			Neuro-/immunotoxicity	Apel et al. (2017)
PBDE-99[b]	BE	Serum	1.7 ng/mL	0			Neurotoxicity	Krishnan et al. (2011)
PFAS[d]	PFOA + PFNA + PFHxS + PFOS at TWI young women	Serum	6.9 ng/mL	21		34/15/20*	Immunotoxicity	EFSA (2020)
PFOA[d]	HBM I general population	Serum	2 ng/mL	12	12/13	16/6.9/14*	Mixed toxicity	Hölzer et al. (2021)
	HBM II general population	Serum	10 ng/mL	0			Mixed toxicity	Schümann et al. (2021)
	HBM II females child-bearing age	Serum	5 ng/mL	0.3			Developmental toxicity	Schümann et al. (2021)
PFOS[d]	HBM I general population	Serum	5 ng/mL	19	27/14*	25/19/18*	Mixed toxicity	Hölzer et al. (2021)
	HBM II general population	Serum	20 ng/mL	1.7	1.7/1.8	5.5/0.25/0*	Mixed toxicity	Schümann et al. (2021)
	HBM II females child-bearing age	Serum	10 ng/mL	3.6		9.8/1.8/1.4*	Developmental toxicity	Schümann et al. (2021)
DEP	BE (MEP)	Urine	18000 μg/L	0			Body growth	Aylward et al. (2009)
DBP	HBM guidance value (HBM-GV), children (MBP)	Urine	120 μg/L	4.8	5.5/4.5	5.2/4.0/5.8	Reproductive/developmental toxicity	Lange et al. (2021)
	HBM-GV adults/adolescents	Urine	190 μg/L	1.5	1.7/1.3	1.2/1.7/1.4	Reproductive/developmental toxicity	Lange et al. (2021)
BBzP	HBM-GV children (MBzP)	Urine	2000 μg/L	0			Reproductive/developmental toxicity	Lange et al. (2021)
	HBM-GV adults/adolescents	Urine	3000 μg/L	0			Reproductive/developmental toxicity	Lange et al. (2021)
DEHP	HBM-GV children (5-oxo-MEHP+5-OH-MEHP)	Urine	340 μg/L	0.2			Reproductive/developmental toxicity	Lange et al. (2021)
	HBM-GV adults/adolescents	Urine	500 μg/L	0.09			Reproductive/developmental toxicity	Lange et al. (2021)
	HBM-GV children (5cx-MEPP+5-OH-MEHP)	Urine	380 μg/L	0.2			Reproductive/developmental toxicity	Lange et al. (2021)
	HBM GV adults/adolescents	Urine	570 μg/L	0.09			Reproductive/developmental toxicity	Lange et al. (2021)

(*continued on next page*)

Table 4 (*continued*)

Substance (metabolite)	Type of HBM-GV[a]	Matrix	HBM-GV	Participants exceeding HBM-GV (%)	Male/female participants exceeding HBM-GV (%)	Participants in grade 5/8/11 exceeding HBM-GV (%)	Critical effect	Reference for HBM-GV
DPHP	HBM GV adults (OH-MPHP)	Urine	220 μg/L	0			Thyroid toxicity	Lange et al. (2021)
	Children	Urine	140 μg/L	0			Thyroid toxicity	Lange et al. (2021)
DiNP	BE (OH-MiNP + oxo-MiNP + cx-MiNP)	Urine	14700 μg/L	0			Liver toxicity	Hays et al. (2011)
DiNCH	HBM GV children (OH-MINCH + cx-MINCH)	Urine	3000 μg/L	0			Kidney toxicity	Lange et al. (2021)
	Adults/adolescents	Urine	4500 μg/L	0			Kidney toxicity	Lange et al. (2021)
BPA	HMB GV children	Urine	135 μg/L	0			Kidney toxicity	Ougier et al. (2021)
	HMB GV adults	Urine	230 μg/L	0			Kidney toxicity	Ougier et al. (2021)
3-PBA	BE Tier 1	Urine	1.7 μg/L	2.2	2.3/2.5	3.3/1.2/2.9	Neurotoxicity	Aylward et al. (2018)
	BE Tier 2	Urine	87 μg/L	0			Neurotoxicity	Aylward et al. (2018)
TCS	HBM I children	Urine	2000 μg/L	0			Haemato-/spleen toxicity	Apel et al. (2017)
	HBM I adults	Urine	3000 μg/L	0			Haemato-/spleen toxicity	Apel et al. (2017)

[a] HBM I is the threshold for early warning and concern, whereas HBM II is the threshold for an increased risk for adverse health effects.
[b] Recalculated from concentration in blood lipids to concentration in serum using a mean lipid content in RMA serum of 0.33%. *$p \leq 0.05$, Chi-square test.
[c] Non-dioxin like.
[d] PFAS ng/g converted to ng/mL using the specific gravity of serum (1.0275) (Sunderman and Boerner, 1949).

Table 5
Hazard index (HI) in RMA (N = 1082) based on the same target organ critical effect.

Target organ[a]	Composition	Total N with hazard index >1 (%)[b]	Median (range) of HI	Median (range)[c] of HI > 1
Liver	HCB, DiNP	35 (3.2)	0.53 (0.15–93)	1.1 (1.01–93)
Kidney	Cd, Pb, DiNCH, BPA	16 (1.5)	0.31 (0.01–4)	1.64 (1.08–4)
Central nervous system	Hg, Pb, Al, NDL[d]-PCB, PBDE-99, 3-PBA	1018 (94)	1.82 (0.58–15)	1.86 (1–15)

[a] Critical effect target for HBM-GVs as seen in Table 4.
[b] HI calculated per target organ critical effect by dividing participants blood/serum/urine concentrations with corresponding HBM-GV (hazard ratio, HR) from Table 4, then summing HR together. Total numbers of those with HI > 1 shown.
[c] Median and range calculated for participants with HI > 1.
[d] Non-dioxin-like.

substances', the dibutyl phthalate (DBP) metabolite monobutyl phthalate (MBP) and the butylbenzyl phthalate (BBzP) metabolite MBzP formed a cluster suggesting common exposure sources (Fig. 1). MBP and MBzP were also moderately correlated with the DEHP metabolites (Fig. S4, Supplement), as correspondingly observed for MBzP among Swedish and Danish children, and for MBP among Flemish adolescents (Bastiaensen et al., 2021; Langer et al., 2014; Larsson et al., 2014). Common exposure from building materials/consumer products may contribute to the observed relations (KEMI, 2015). As among Swedish and Danish children, and Flemish adolescents (Bastiaensen et al., 2021; Langer et al., 2014; Larsson et al., 2014), in RMA only negligible/weak correlations were observed between the metabolite of diethyl phthalate (DEP), monoethyl phthalate (MEP), and the other phthalate metabolites (Fig. S4, Supplement). DEP is currently the main phthalate used in cosmetic products in the EU (KEMI, 2015). DiNP, diiso-decyl phthlate (DiDP) and dipropylheptyl phthalate (DPHP) have been introduced on the European market more recently than the other phthalates (Fréry et al., 2020), and the metabolites of these three phthalates were moderately correlated with each other (Fig. S4, Supplement). DiNP and DPHP metabolites were also moderately correlated with the DEHP metabolites (Fig. S4, Supplement), also observed for DiNP and DEHP metabolites in Flemish adolescents (Bastiaensen et al., 2021). DiNP is a direct alternative to DEHP as a general plasticizer and are used together with both DiNP and DPHP in PVC production (KEMI, 2015). The two PAH metabolites 2-hydroxy-phenanthrene (2-OH-PH) and 1-HP formed a cluster and were moderately correlated (Fig. 1 & Fig. S4, Supplement), as expected due to the common exposure sources (Murawski et al., 2020)

3.2. Comparisons with other studies

Comparisons of RMA results with other studies of adolescents are somewhat uncertain due to differences in study design/populations and analytical methods. Nevertheless, mean concentrations of elements, POPs and urine substances with a few exceptions varied less than 3-fold between RMA against NHANES (USA), GerES (Germany) and FLEHS (Flanders, Belgium) (Tables 2 and 3). The relatively small differences in average exposure of adolescents between the four studies are most probably due to the similarly high standard of living in these populations, in combination with world-wide distribution of chemicals and products. NHANES showed large ethnic differences in mean serum concentrations of chlorinated and brominated POPs, in some cases up to several orders of magnitude (Table 2) (NHANES, 2022). In RMA, mean concentrations of chlorinated POPs were within these ranges or somewhat lower (Table 2). The concentrations of the three PBDEs were in most cases below the LOQ (<20 pg/ml serum) in RMA (Table S3, supplement). However, the determined concentrations below LOQ in RMA strongly suggested considerably lower mean serum PBDE concentrations than in NHANES, with a minimum of a 23-fold difference (BDE-153) (Table 2) (NHANES, 2022). The higher body burdens of PBDE in the US than in Sweden and other European countries is in line with studies of adults (Fromme et al., 2016). Differences in PBDE legislation between the US and Europe most likely contributes to the exposure differences (Horton et al., 2013). The mean concentration of

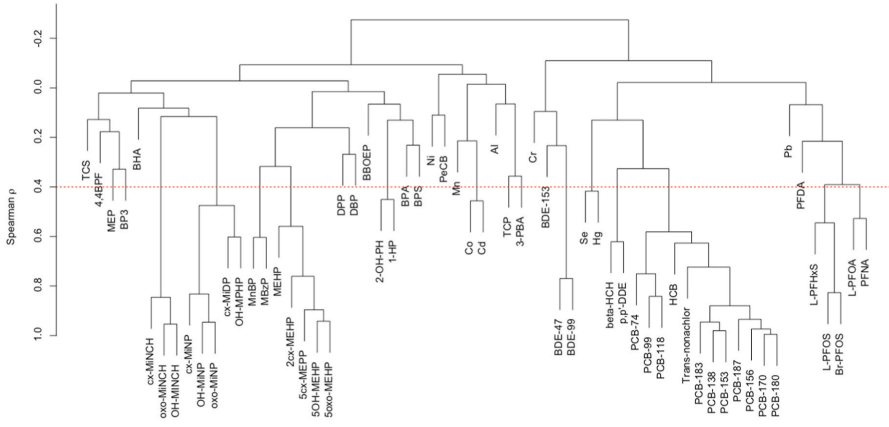

Fig. 1. Cluster analyses of correlations between toxic substance concentrations among adolescents in RMA (N = 1082). Correlation coefficients should be read at where branches separate and not on the names. Figure shows absolute spearman correlation coefficients with both negative and positive correlations. The dotted red line represents the set cut-off for moderate correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

dichlorodiphenyltrichloroethane (p,p'- DDT) was reported to be 37-fold higher in FLEHS compared to RMA, but the concentration of the DDT metabolite p,p-DDE did not differ markedly (Table 2). Studies of breast milk suggest similar average p,p'-DDT exposures among women in child-bearing age in Sweden and Belgium around the same time period as the RMA study (Aerts et al., 2019; Kallerman et al., 2021). Further studies are needed to determine the reason behind the large differences in p,p'-DDT concentrations in RMA and FLEHS.

The average concentrations of phthalate metabolites in urine did in many cases not differ more than 3-fold between RMA and the other studies, suggesting similar exposure levels among the adolescents (Table 3). Among the European adolescents this may be due to the common plastics regulation within the EU (CEP et al., 2022; Pereira et al., 2022). Nevertheless, the exceptions to the <3-fold difference were MBP, cx-MiNP, cx-MiDP and 6-hydroxy monopropylheptyl phthalate (OH-MPHP). The mean MBP concentrations in NHANES, GerES and FLEHS were lower than in RMA (Table 3), at most being 3.6-fold lower in NHANES compared with RMA. Similarly, the mean cx-MiNP was also lower in GerES and FLEHS (not reported in NHANES), occurring at a 4.2-fold lower mean concentration in FLEHS. However, the differences in mean concentrations of the other DiNP metabolites were less than 3-fold and RMA did not consistently show the highest concentrations (Table 3). cx-MiDP was lowest in RMA, at most 5.4-fold lower than in NHANES (Table 3), and OH-MPHP was 3.7-fold lower in GerES than in RMA. There was no consistent pattern in the observed differences in mean concentrations of the four phthalate metabolites between RMA and the other three studies, suggesting diverging substance-related exposure sources. The largest differences of concentrations of urine substances were observed for TCS and BP3, being on average about 15-fold higher in NHANES adolescents compared to RMA (Table 3). Although concentrations of these substances were below LOQ in GerES, they were also clearly lower than in NHANES. Differences in the levels of TCS and BP3 in consumer products and also in usage patterns, between Germany and the US were suggested as explanations of divergence in TCS exposure between GerES and NHANES (Tschersich et al., 2021), and is likely also the reason for the large difference between RMA and NHANES.

3.3. Comparisons with HBM-GVs and HI assessment

We surveyed the literature for HBM-GVs and found several from multiple sources (Table 4). There are uncertainties when comparing the different proposed HBM-GV, in part due to variation in the quality of the toxicological database, in procedures used in the development of the different values, safety margins used between point-of-departure of critical effect concentrations and HBM-GVs, and the fact that some of them have not been updated based to the most recent risk assessments.

Moreover, some HBM-GVs listed in Table 4 were specifically developed for distinct sub-populations like children, adolescents, adults or women in childbearing age. UBA, Germany (see Table 4 references) developed HBM I and HBM II values, and in the view of UBA there is no risk of adverse health effects if concentrations of substances are below HBM I. If the concentrations are above HBM I, but below HBM II, efforts should be made to search for potential sources of exposure and to mitigate identified exposure sources. An exceedance of HBM II is by UBA regarded as an increased health risk, and therefore there is a need for acute action to reduce exposure and biomedical advice should also be provided. Biomonitoring Equivalent (BE) values are based on existing health-based guidance values, such as tolerable intakes, and exceedances of BE may be regarded as a health concern (Hays et al., 2007). HBM4EU has developed HBM-GVs that in reality are equivalent to UBA's HBM I values (Apel et al., 2020). The values for Pb, Al, and $PFAS_4$ (Table 4) were based on benchmark concentrations/effect level concentrations in humans without any safety factors (EFSA, 2020; Riihimäki et al., 2000). Moreover, the Al value was based on relationships between occupational exposure and health among aluminium welders (Riihimäki et al., 2000). Despite all these uncertainties, as a conservative measure, exceedances of the HBM-GVs among the RMA participants were regarded as indications of a health concern.

The largest degree of exceedance of HBM I, HBM-GVs for children, and benchmark concentrations/effect levels were observed for Al, $PFAS_4$, PFOS, PFOA and Pb in declining order, all being ≥12% of the adolescent population when looking at the most conservative values (Table 4). Based on the size of the adolescent population in Sweden during 2016 for ages 12, 15 and 18 (N = 324776) (SCB, 2022), the number of adolescents exceeding the HBM-GVs can be estimated to range from 32700 ($PFAS_4$, females only) to 84400 (Al). For HCB, MBP and 3-PBA percent exceedances corresponded to 10000, 16300 and

7100 adolescents, respectively (Table 4). The percentages of exceedances of PFASs are likely overestimated since 5% of the participants were living in areas with a history of PFAS contamination of drinking water (Nyström et al., 2022), which is disproportionally high from a nation-wide population perspective. To the best of our knowledge, the PFAS contamination in some areas of Sweden is a special case not representative for the other substances included in our study. Nevertheless, contribution of unknown local hotspot contamination of other substances to the exceedances of HBM-GVs cannot be excluded. Exceedances were also observed for Cd, Hg, DEP, and DEHP, although to a lesser degree (Table 4). Overall, 54% of the participants exceeded at least one of the HBM-GV, 18% at least two, and 5% at least three. Taken together the results show that a significant fraction of the adolescent population had overall high exposures to several of the toxic substances in relation to the HBM-GVs. When using HBM-GVs for adults and/or adolescents (Pb, DBP, DEHP) or HBM II values (Hg, PFOS) the percentages exceeding the HBM-GVs were less frequent (Table 4). Considering that the concentrations of toxic substances in RMA, GerEs V and FLEHS were comparable (Tables 2 and 3), it is not surprising that GerES and FLEHS also reported exceedances of HBM-GVs of Cd, Pb (FLEHS), Hg (GerES), HCB (FLEHS), PFASs, DBP, DEHP (GerES) and 3-PBA (FLEHS) (Schwedler et al., 2020b; Vogel et al., 2021).

As suggested by the higher GM concentrations of Pb, HCB, and PFOS among males than females in RMA (Table 1), fewer females exceeded the benchmark dose of 1% extra risk (BMDL1%) for neurotoxicity of Pb in children, the BE for HCB, and the HBM I value for PFOS (Table 4). However, observed gender differences in concentrations of the toxic substances (Table 1) did not necessarily result in gender differences in exceedances of HBM-GVs, as observed for PFOA, the DBP metabolite MBP, and 3-PBA (Table 4). Moreover, no gender differences in exceedances of HBM-GVs were observed for Al, and for less conservative HBM-GVs of Pb, PFOS, and MBP. When looking at age-dependent differences in exceedances in relation to observed associations between age and concentrations there were no general trend, except in the case of PFASs that showed a higher degree of exceedances among 5th graders (Table 4), most probably due to a particular subset of the 5th-grade participants (N = 58) having a known high exposure of PFHxS and PFOS from drinking water in Uppsala and Ronneby (Nyström et al., 2022).

Efforts to eliminate environmental pollution of Pb, HCB, PFOA, PFNA, PFHxS and PFOS have resulted in slowly declining temporal trends in different Swedish populations (Gyllenhammar et al., 2021; Lundh et al., 2020; Miaz et al., 2020; Norén et al., 2021; Wennberg et al., 2017). Similar efforts to reduce Cd pollution have not resulted in declining Cd exposures during the last few decades, whilst mixed trends for Hg exposure (no decrease/slow decrease) have been reported (Kippler et al., 2021; Lundh et al., 2020; Wennberg et al., 2017). Taken together, further efforts to reduce exposures to Pb, Hg, Cd, Al, HCB and PFASs in Sweden are clearly needed. A study of young women in Sweden have reported decreasing exposures to DEP, DBP and DEHP over the last few decades, suggesting that the exposure situation also is slowly improving for these rapidly metabolized substances (Gyllenhammar et al., 2017).

In accordance with the RMA findings of exceedances of HBM-GVs of Pb, Hg (methyl-Hg), Al, and PFASs, The European Food Safety Authority (EFSA) concluded that the tolerable intakes of these substances from the diet were exceeded by parts of the EU population at the time of risk assessment (EFSA, 2012a, 2012b, 2010, 2008). Regarding phthalates, EFSA stated that the group tolerable intake of DBP, BBP, DEHP and DiNP (as DEHP equivalents) were not exceeded by EU populations even when considering the worst-case total dietary intake mainly originating from plastic food contact materials (CEP et al., 2019). However, diet is only one of several potential exposure sources of these phthalates, and the contribution of the additional sources most likely contributes to the high concentrations of metabolites observed in a small fraction of the RMA participants (Bastiaensen et al., 2021; Langer et al., 2014; Larsson et al., 2014; Schwedler et al., 2020b). However, considering that RMA only included single-spot urine sample there are still uncertainties about the risks of long-term individual exceedances of the HBM-GVs for these phthalates among adolescents in Sweden.

BPA concentrations in urine did not exceed the published HBM-GV, the GM concentration being 148-fold lower than the HBM-GV for children (Tables 1 and 4). However, in 2021 an EFSA draft opinion on health risks of BPA in foods proposed a considerably lower tolerable intake compared to the pervious assessment in 2015 (EFSA, 2021). It was proposed that populations with both average and high exposures to BPA in all age groups exceed the new tolerable intake. The publication of the final risk assessment will reveal if the exposure of the Swedish adolescent population will be regarded as too high or not from a health risk assessment perspective.

In the cumulative health risk assessment liver toxicity, including HCB and DiNP, showed a median HI (range) of 0.53 (0.15–93), with 3.2% of the participants showing a HI > 1 (Table 5). HCB was the driver of the health concern contributing with 99.9% (median, range: 98.8–100%) to the HI among participants with HI > 1. As with HCB alone, the highest proportion of participants with a health concern HCB was observed among males (5.9%), with a significantly lower proportion among females (1.2%, Chi-square test $p \leq 0.05$). In the case of kidney toxicity, the median HI (range) was estimated to 0.31 (0.006–4.0) and 1.5% of the participants had a HI > 1 (Table 5). Cd contributed 76.3% (3.1–91%) to the HI among participants with HI higher than 1, followed by Pb 23% (7.7–96%). The proportion of males and females with a HI higher than 1 was 1.6% and 1.3%, respectively, with gender difference not being statistically significant. Neurotoxic substances consisting of Hg, Pb, Al, NDL-PCB, PBDE-99 and 3-PBA showed a higher median HI of 1.8 (range: 0.58–15) compared to substances with liver and kidney toxicity, with 94% of the participants showing a health concern (HI > 1). Al contributed the most 39.5% (0–93%) to the HI among participants with HI higher than 1, followed by Pb 33.8% (0–96%). As with liver and kidney toxicity males showed a higher proportion of 96% exceeding HI = 1, whereas the proportion of females was 93% (Chi-square test $p \leq 0.05$). The HI analysis indicates that if the assumption of cumulative additive target organ toxicity effect holds true, significant portions of the adolescent population may be at risk of having too high cumulative exposures to neurotoxic substances. However, granted that the target organ of the central nervous system (CNS) is highly complex and the neurotoxicity of each individual contributing substance may affect different sections or processes of the CNS, the observation should be considered preliminary.

3.4. Strengths and limitations

As previously eluded to, a major strength of this nation-wide population-based study of adolescents is the inclusion of wider groups of toxic substances. There are some minor demographic/life-style differences between the RMA participants and the adolescent population in Sweden, yet participants are still considered as representative of the adolescent population in Sweden with regard to school type/size, and parental education and income (Moraeus et al., 2018). The urine substances have very short half-lives in comparison to the elements and POPs. As a consequence there is intra-individual variation of urine substance concentrations depending on timing of sampling. Other studies have shown that there may be seasonal differences in concentrations for some of the substances in urine (Bastiaensen et al., 2021). Although the possibility of seasonal differences in concentrations were not analysed statistically in the present study, the present results may be regarded as representative for the general adolescent population in Sweden during the sampling period. While aspects of the present work are comparable to prior studies (e.g. NHANES and GerES) some caution is warranted when comparing between studies due to potential differences in study design, analytical methods, and data processing/analyses. Human biomonitoring initiatives of toxic substances has been increasingly moving

towards combination and mixture effects. Yet, most studies still only focus on single substances/substance groups. Inclusion of a wider portion of exposome as in the present study, uncovered patterns of chemical body burdens that are valuable for interpretation of future RMA health studies. In the analyses of urine substances, the precision of cx-MiDP, BPS, DBP and BHA was >20%. This introduced an additional uncertainty in the statistical analyses, and in comparisons with other studies and HBM-GV.

The HBM4EU initiative, as well as UBA, has made steady progress to developing HBM-GVs for the general population (Apel et al., 2020), but nevertheless many toxic substances currently lack established HBM-GVs. We attempted to include the HBM-GVs appropriate for a general adolescent population, but if unavailable we instead used either those developed for children/adults or values proposed for occupational exposure (Al), making comparison of the results uncertain. Moreover, there are uncertainties connected to our limited HI approach of grouping of substances with the same target critical effect organs, and the HI results therefore should be considered as hypothesis generating for future studies of possible combination effects of the included chemicals.

4. Conclusion

Although many biomonitoring studies are available involving a range of individual chemical substances, our study is one of the few attempts at looking at the wider chemical exposome within a single study of adolescents, giving a more holistic and clearer picture of the current state of the chemical body burden in Swedish adolescents. With few exceptions, chemical body burdens in the present work were similar to those observed in adolescents from Germany and the US, showing a similar exposome for adolescents in these economically and industrially developed countries from two different continents. In RMA, the moderate to strong correlations between substances within the same chemical groupings were most likely due to common sources of exposure and/or similar toxicokinetics. However, while the chlorinated, brominated and fluorinated POPs included here share common properties of environmental persistence and bioaccumulation in humans, measured concentrations of the different contaminant classes were rarely correlated. Moreover, no correlations were generally observed between substances measured in different matrices. These observations are important for better interpretation of results in future evaluations of RMA data with regards to possible combination effects. The gender patterns observed, with generally higher average body burdens of neurotoxic Pb, Hg and some POPs in males compared to females, reflected in males having higher proportions of HI $>$ 1 for neurotoxicity also adds important knowledge for future combination effect studies. Our results give support to further efforts to reduce human exposures to toxic chemicals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114196.

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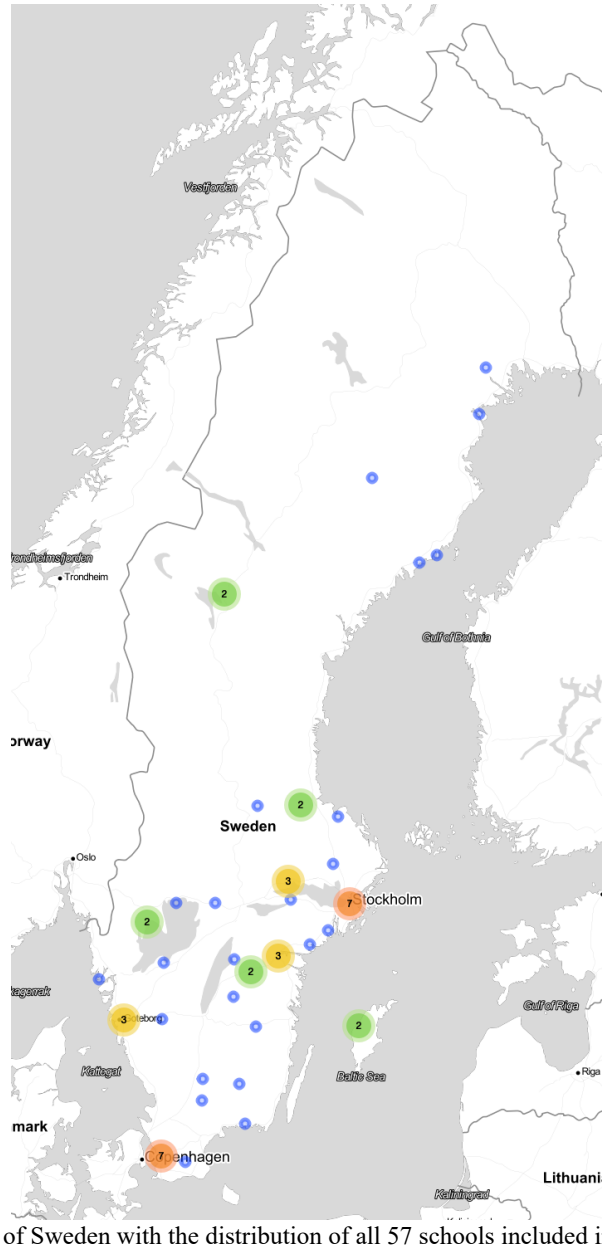

Figure S1. Map of Sweden with the distribution of all 57 schools included in this study, 2016-17. Single schools are indicated by a blue dot. Schools that were within city-wide proximity to each other are presented clustered together via numbered coloured circles corresponding to the number of schools, were Orange > Yellow > Green = 7 > 3 > 2; Sweden encompasses latitudes ~55° to ~69° north, and longitudes ~11° to 25° east, with the largest urban areas in the south-west being Malmö (latitude 55.6°, longitude 13°) and Gothenburg (57.7°, 11.9°) along with Stockholm (59.3°, 18°) in the east.

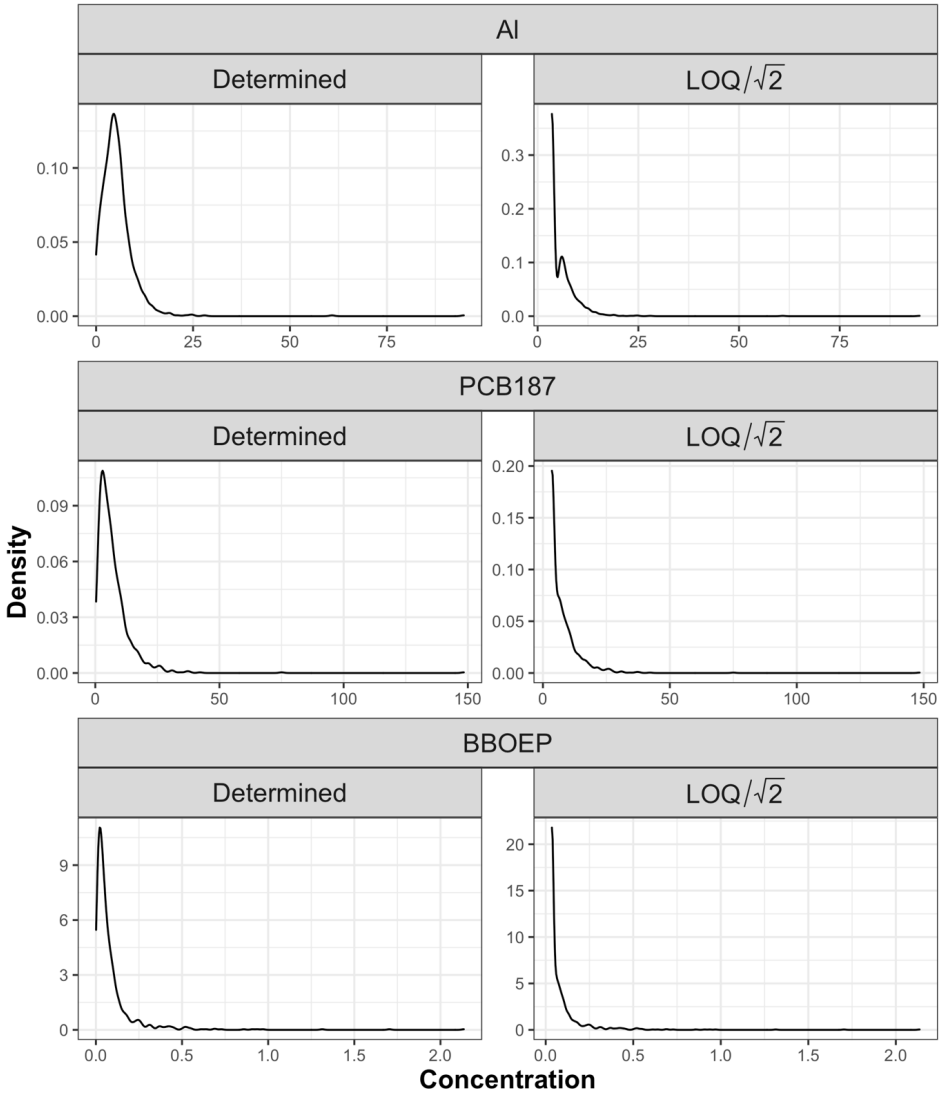

Figure S2. Change in concentration distribution of the substances Al, PCB-187 and BBOEP after replacement of concentrations <LOQ or LOD with determined concentrations instead of fixed concentrations at LOQ or LOD/√. "Determined" shows plots where the determined concentrations below LOQ or LOD have been used (see section Handling of data below LOQ/LOD in the article), whereas "LOQ/√2" shows plots where concentrations below LOQ or LOD have been set to a concentration at LOQ/√2. Distribution of concentrations using determined concentrations, although right-skewed, shows a better distribution curve than distributions using LOQ/√2 concentrations.

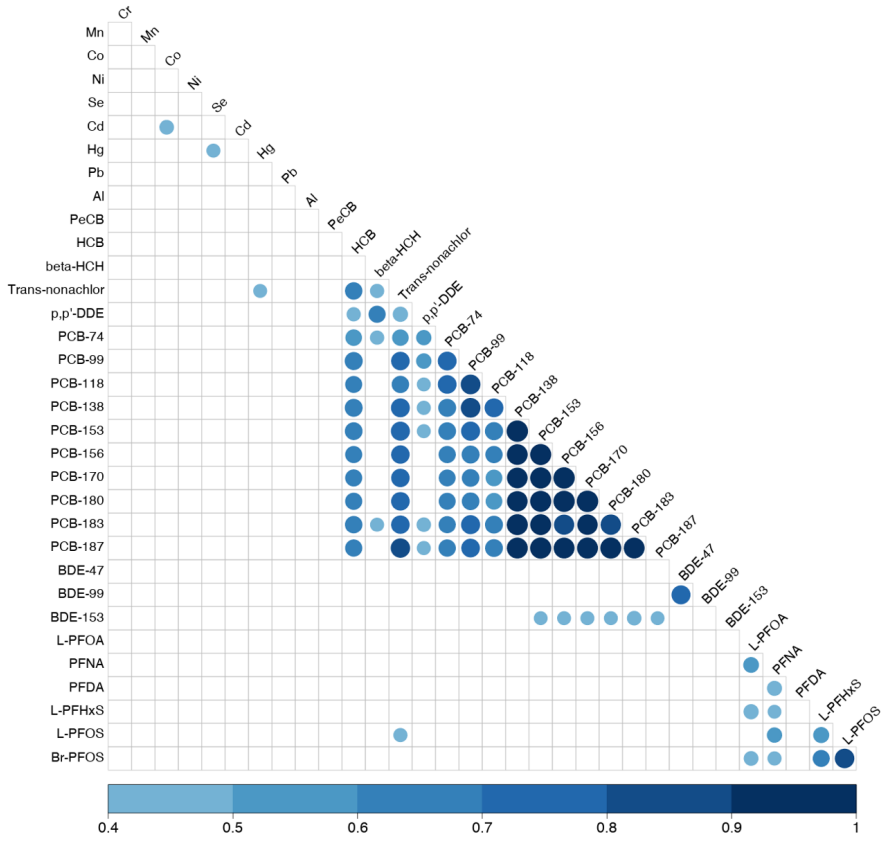

Figure S3. Spearman's correlation coefficient matrix showing correlations between substances analysed in whole blood and serum. Increasing size and colour density of the circles corresponds to substances moderately-highly correlated ($\rho \geq 0.40$). Correlation coefficients >0.061 were statistically significant ($p \leq 0.05$). None of the negative spearman correlations were below -0.4, therefore only positive correlations are shown.

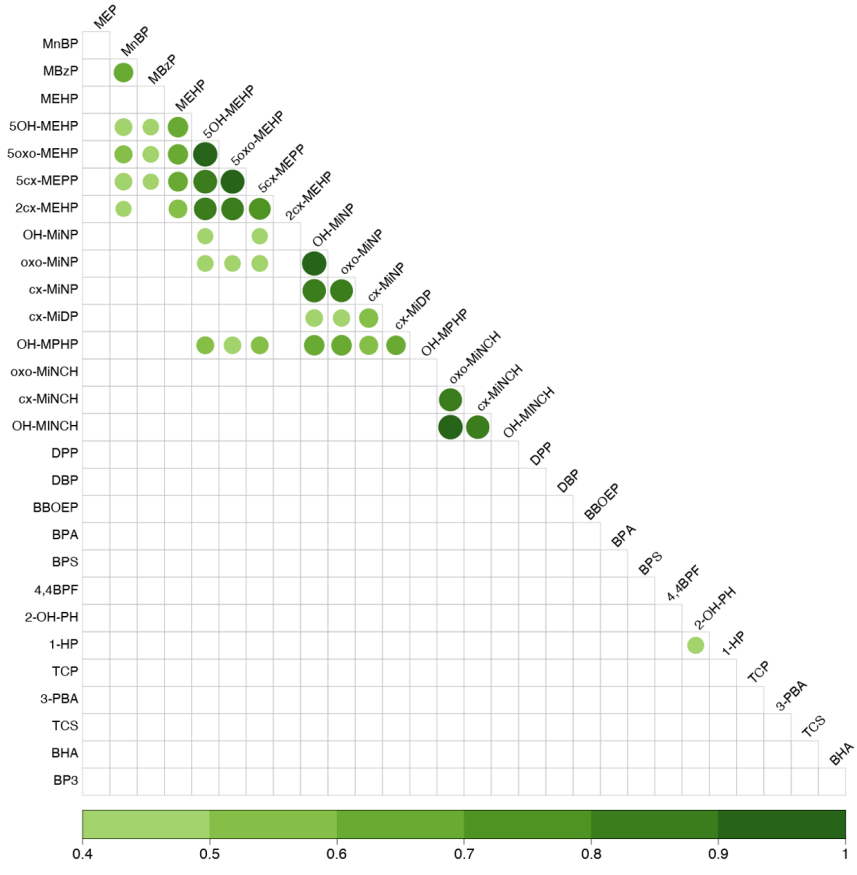

Figure S4. Spearman's correlation coefficient matrix showing correlations between substances analysed in urine. Increasing size and colour density of the circles corresponds to substances moderately-highly correlated ($\rho \geq 0.40$). Correlation coefficients >0.061 were statistically significant ($p \leq 0.05$). None of the negative spearman correlations were below -0.4, therefore only positive correlations are shown.

Table S1. School grade-related differences in toxic substance concentrations in RMA[a].

Substances[b]		Geometric mean (95% CI)			p-value[c]
Abbreviation	Unit / Matrix	5th Grade (N=329)	8th Grade (N=405)	11th Grade (N=348)	
Cr	µg/L whole blood	0.55 (0.53 - 0.56)	0.56 (0.54 - 0.57)	0.59 (0.57 - 0.62)	<0.001
Mn	µg/L whole blood	10.3 (9.77 - 10.8)	11.3 (11.0 - 11.6)	10.0 (9.70 - 10.3)	<0.001
Co	µg/L whole blood	0.11 (0.11 - 0.12)	0.15 (0.14 - 0.16)	0.11 (0.10 - 0.12)	<0.001
Ni	µg/L whole blood	0.61 (0.60 - 0.63)	0.64 (0.63 - 0.66)	0.61 (0.59 - 0.63)	0.001
Se	µg/L whole blood	89.4 (85.6 - 93.3)	95.3 (94.0 - 96.6)	99.8 (98.3 - 101.4)	<0.001
Cd	µg/L whole blood	0.09 (0.09 - 0.10)	0.13 (0.12 - 0.13)	0.16 (0.14 - 0.17)	<0.001
Hg	µg/L whole blood	0.69 (0.63 - 0.75)	0.66 (0.61 - 0.72)	0.63 (0.57 - 0.69)	0.336
Pb	µg/L whole blood	7.42 (6.98 - 7.88)	7.32 (6.98 - 7.67)	7.24 (6.89 - 7.61)	0.288
HCB	pg/mL serum	42.9 (41.1 - 44.8)	43.4 (41.5 - 45.3)	40.1 (38.6 - 41.7)	0.013
p,p'-DDE	pg/mL serum	105 (95.8 - 115)	109 (100 - 117)	115 (104 - 127)	0.399
PCB-118	pg/mL serum	6.45 (6.07 - 6.86)	6.65 (6.32 - 6.99)	6.90 (6.51 - 7.33)	0.181
PCB-138	pg/mL serum	24.5 (22.7 - 26.4)	27.5 (25.8 - 29.3)	26.3 (24.6 - 28.2)	0.054
PCB-153	pg/mL serum	38.5 (35.5 - 41.7)	45.0 (42.1 - 48.1)	41.4 (38.5 - 44.6)	0.012
PCB-170	pg/mL serum	10.1 (9.16 - 11.1)	12.5 (11.6 - 13.6)	11.5 (10.6 - 12.5)	0.001
PCB-180	pg/mL serum	20.0 (18.2 - 22.0)	25.3 (23.4 - 27.5)	23.1 (21.2 - 25.1)	0.001
PCB-187	pg/mL serum	4.54 (4.13 - 5.00)	5.69 (5.25 - 6.16)	5.17 (4.75 - 5.63)	0.003
L-PFOA	ng/g serum	1.34 (1.27 - 1.41)	1.12 (1.08 - 1.17)	1.21 (1.15 - 1.27)	<0.001
PFNA	ng/g serum	0.37 (0.35 - 0.40)	0.34 (0.32 - 0.36)	0.38 (0.35 - 0.40)	0.006
PFDA	ng/g serum	0.15 (0.14 - 0.16)	0.15 (0.14 - 0.15)	0.16 (0.14 - 0.17)	0.511
L-PFHxS	ng/g serum	0.74 (0.63 - 0.86)	0.38 (0.35 - 0.41)	0.36 (0.33 - 0.39)	<0.001
L-PFOS	ng/g serum	2.55 (2.32 - 2.80)	1.94 (1.83 - 2.06)	2.01 (1.88 - 2.14)	<0.001
br-PFOS	ng/g serum	1.22 (1.10 - 1.35)	0.85 (0.80 - 0.90)	0.89 (0.83 - 0.95)	<0.001
MEP	ng/mL urine	28.8 (25.5 - 32.5)	42.1 (37.9 - 46.7)	56.8 (50.2 - 64.4)	<0.001
MBP	ng/mL urine	43.3 (40.3 - 46.5)	40.8 (38.5 - 43.2)	40.7 (37.7 - 43.8)	0.334
MBzP	ng/mL urine	7.64 (6.83 - 8.54)	5.80 (5.21 - 6.46)	8.09 (7.18 - 9.11)	<0.001
MEHP	ng/mL urine	1.91 (1.76 - 2.07)	1.53 (1.43 - 1.64)	1.81 (1.69 - 1.95)	<0.001
5OH-MEHP	ng/mL urine	9.95 (9.21 - 10.7)	7.50 (7.04 - 7.99)	7.07 (6.48 - 7.71)	<0.001
5oxo-MEHP	ng/mL urine	7.86 (7.27 - 8.51)	5.79 (5.42 - 6.18)	5.83 (5.39 - 6.31)	<0.001
5cx-MEPP	ng/mL urine	9.21 (8.52 - 9.95)	6.69 (6.26 - 7.15)	6.45 (5.98 - 6.96)	<0.001
2cx-MEHP	ng/mL urine	2.53 (2.34 - 2.74)	1.97 (1.83 - 2.11)	1.96 (1.83 - 2.10)	<0.001
OH-MiNP	ng/mL urine	4.49 (4.06 - 4.98)	4.38 (3.94 - 4.87)	5.01 (4.48 - 5.61)	0.106
oxo-MiNP	ng/mL urine	2.09 (1.90 - 2.29)	2.04 (1.85 - 2.24)	2.37 (2.14 - 2.62)	0.028
cx-MiNP	ng/mL urine	6.82 (6.18 - 7.52)	6.87 (6.22 - 7.58)	7.89 (7.07 - 8.81)	0.115
cx-MiDP	ng/mL urine	0.42 (0.39 - 0.46)	0.38 (0.35 - 0.41)	0.42 (0.39 - 0.46)	0.041
OH-MPHP	ng/mL urine	1.11 (1.03 - 1.21)	1.09 (1.01 - 1.19)	1.16 (1.06 - 1.27)	0.605
oxo-MiNCH	ng/mL urine	1.15 (1.02 - 1.30)	1.21 (1.09 - 1.35)	1.53 (1.33 - 1.77)	0.031
cx-MINCH	ng/mL urine	0.82 (0.73 - 0.94)	0.91 (0.81 - 1.02)	1.14 (0.98 - 1.33)	0.062
OH-MINCH	ng/mL urine	0.86 (0.75 - 0.98)	0.92 (0.82 - 1.04)	1.11 (0.96 - 1.30)	0.173
DPP	ng/mL urine	1.96 (1.81 - 2.11)	1.97 (1.85 - 2.10)	2.04 (1.89 - 2.20)	0.604
DBP	ng/mL urine	0.17 (0.15 - 0.19)	0.14 (0.13 - 0.15)	0.14 (0.13 - 0.16)	0.015
BPA	ng/mL urine	0.94 (0.85 - 1.04)	0.77 (0.70 - 0.84)	0.97 (0.86 - 1.08)	<0.001
BPS	ng/mL urine	0.14 (0.13 - 0.16)	0.13 (0.12 - 0.14)	0.17 (0.15 - 0.19)	<0.001
4,4BPF	ng/mL urine	0.06 (0.05 - 0.08)	0.11 (0.09 - 0.13)	0.16 (0.13 - 0.19)	<0.001
2-OH-PH	ng/mL urine	0.14 (0.12 - 0.15)	0.18 (0.16 - 0.20)	0.19 (0.17 - 0.21)	<0.001
TCP	ng/mL urine	1.29 (1.19 - 1.39)	1.23 (1.16 - 1.31)	1.39 (1.29 - 1.50)	0.109
3-PBA	ng/mL urine	0.30 (0.28 - 0.33)	0.28 (0.26 - 0.30)	0.27 (0.24 - 0.29)	0.156
TCS	ng/mL urine	0.23 (0.20 - 0.26)	0.31 (0.27 - 0.35)	0.36 (0.31 - 0.42)	<0.001
BHA	ng/mL urine	0.35 (0.25 - 0.49)	0.68 (0.50 - 0.92)	1.17 (0.84 - 1.63)	<0.001
BP3	ng/mL urine	0.58 (0.50 - 0.67)	1.18 (0.99 - 1.41)	1.63 (1.36 - 1.96)	<0.001

[a] Age of participants 5th grade: median 11 years (range: 10-13), 8th grade: 14 years (11-15) and 11th grade: 17 years (16-21).

[b] Substances with over 50% of samples <LOQ or LOD are not included in this table. For the purpose of calculating geometric means and 95% confidence intervals (CI), determined concentrations <LOQ or <LOD were used and concentrations determined to be 0 were converted to the lowest concentration above 0/sqrt(2) (see section Handling of data below LOQ/LOD). PFASs concentrations <LOD were converted to LOD/sqrt(2), else concentrations <LOQ to LOQ/sqrt(2).

[c] Kruskal-Wallis test on comparison of concentrations by school grade. p-values <0.05 indicate significant difference in concentrations in at least one grade.

Table S2. Substance abbreviation and full name, CAS number, industrial/consumer use.

Substance abbreviation	Substance full name	CAS #	Industrial use
Substances detected in whole blood			
Cr	Chromium	7440-47-3	Metal industry, building materials, consumer products
Mn	Manganese	7439-96-5	Metal industry, building materials, consumer products
Co	Cobalt	7440-48-4	Batteries, metal industry, building materials, consumer products
Ni	Nickel	7440-02-0	Metal industry, building materials, consumer products
Se	Selenium	7782-49-2	Building materials, electric equipment, consumer products
Cd	Cadmium	7440-43-9	Metal industry, batteries, electric and electronic equipment
Hg	Mercury	7439-97-6	Dental materials, electric/electronic/medical equipment
Pb	Lead	7439-92-1	Batteries, electronics, ammunition
Substances detected in serum			
Al	Aluminium	7429-90-5	Metal industry, manufacturing material, consumer products
PeCB	Pentachlorobenzene	608-93-5	Fungicide, chemical industry
HCB	Hexachlorbenzene	118-74-1	Fungicide, fireworks
HCH	**Hexachlorocyclohexane**		**Insecticide**
α-HCH	alpha-Hexachlorocyclohexane	319-84-6	Component of HCH
β-HCH	beta-Hexachlorocyclohexane	319-85-7	Component of HCH
γ-HCH	gamma-Hexachlorocyclohexane	58-89-9	Component of HCH and lindane
Chlordane	**Chlordane**		**Insecticide**
Oxychlordane	Oxychlordane	27304-13-8	Metabolite of chlordane
trans-Nonachlor	trans-Nonachlor	39765-80-5	Chlordane component
DDT	**Dichlorodiphenyltrichloroethane**		**Insecticide**
p,p'-DDT	1,1-bis-(4-chlorophenyl)-2,2,2-trichlorethane	50-29-3	Component of insecticide DDT
p,p'-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene	75-55-9	Metabolite of DDT
PCB	**Polychlorinated biphenyls**		**Industrial chemical**
PCB-74	2,4,4',5-Tetrachlorobiphenyl	32690-93-0	PCB congener
PCB-99	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	PCB congener
PCB-118	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	PCB congener
PCB-138	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	PCB congener
PCB-153	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	PCB congener
PCB-156	2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4	PCB congener
PCB-170	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	PCB congener
PCB-180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	PCB congener
PCB-183	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	PCB congener
PCB-187	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	PCB congener
PBDE	**Polybrominated diphenylethers**		**Flame retardant**
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	PBDE congener
BDE-99	2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	PBDE congener
BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	PBDE congener
PFAS	**Per- and polyfluoroalkyl substances**		**Surfactant in products**
L-PFOA	Linear Perfluorooctanoic acid	335-67-1	PFOA isomer
PFNA	Perfluorononanoic acid	375-95-1	PFAS homologue
PFDA	Perfluorodecanoic acid	335-76-2	PFAS homologue
PFUnDA	Perfluoroundecanoic acid	2058-94-8	PFAS homologue
L-PFHxS	Linear Perfluorohexanesulfonic acid	355-46-4	PFHxS isomer

Substance abbreviation	Substance full name	CAS #	Industrial use
L-PFOS	Linear Perfluorooctanesulfonic acid	1763-23-1	PFOS isomer
br-PFOS	Branched Perfluorooctanesulfonic acid	1763-23-1	PFOS isomer
Substances detected in urine			
DEP	**Diethyl phthalate**	**84-66-2**	**Plasticizer**
MEP	Monoethyl phthalate	2306-33-4	Metabolite of DEP
DBP	**Dibutyl phthalate**	**84-74-2**	**Plasticizer**
MBP	Monobutyl phthalate	131-70-4	Sum of DEP metabolites
BBzP	**Butylbenzyl phthalate**	**85-68-7**	**Plasticizer**
MBzP	Monobenzyl phthalate	2528-16-7	Metabolite of BBzP
DEHP	**Di-2-ethylhexyl phthalate**	**117-81-7**	**Plasticizer**
MEHP	Mono(2-ethylhexyl) phthalate	4376-20-9	Metabolite of DEHP
5-OH-MEHP	Mono-(2-ethyl-5-hydroxyhexyl) phthalate	40321-99-1	Metabolite of DEHP
5-oxo-MEHP	Mono-(2-ethyl-5-oxohexyl) phthalate	40321-98-0	Metabolite of DEHP
5-cx-MEPP	Mono-(2-ethyl-5-carboxypentyl) phthalate	40809-41-4	Metabolite of DEHP
2-cx-MEHP	Mono(2-(carboxymethyl-hexyl) phthalate	82975-93-7	Metabolite of DEHP
DiNP	**Di-isononyl phthalate**	**28553-12-0**	**Plasticizer**
OH-MiNP	Mono-(4-methyl-7-hydroxyoctyl) phthalate	936021-98-6	Metabolite of DiNP
oxo-MiNP	Mono-(4-methyl-7-oxooctyl) phthalate	936022-00-3	Metabolite of DiNP
cx-MiNP	Mono-(4-methyl-7-carboxyheptyl) phthalate	936022-02-5	Metabolite of DiNP
DiDP	**Di-isodecyl phthalate**	26761-40-0	**Plasticizer**
cx-MiDP	Mono-carboxy-isononyl phthalate		Metabolite of DiDP
DPHP	**Di-(2-propylheptyl) phthalate**	**53306-54-0**	**Plasticizer**
OH-MPHP	6-Hydroxy monopropylheptyl phthalate	1372605-11-2	Metabolite of DPHP
DiNCH	**Diisononyl-cyclohexane-1,2-dicarboxylate**	**166412-78-8**	**Plasticizer**
cx-MINCH	Cyclohexane-1,2-dicarboxylate-mono (7-carboxylate-4-methyl)heptylester		DiNCH metabolite
OH-MINCH	Cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester		DiNCH metabolite
TPP	**Triphenyl phosphate**	**115-86-6**	**Flame retardant, plasticizer**
DPP	Diphenyl phosphate	838-85-7	Metabolite of TPP
TBP	**Tri-n-butyl phosphate**	**126-73-8**	**Flame retardant, plasticizer**
DBP	Dibutyl phosphate	107-66-4	Metabolite of TBP
TBEP	**Tri(2-butoxyethyl) phosphate**	**78-51-3**	**Flame retardant, plasticizer**
BBOEP	Bis(2-butoxyethyl) phosphate	14260-97-0	Metabolite of TBEP
Bisphenols			**Plastics, epoxy resins, dyes, paint additives, thermal paper, dental sealants**
BPA	Bisphenol A	80-05-7	
BPS	Bisphenol S	80-09-1	
4,4-BPF	4,4-Bisphenol F	620-92-8	
PAH	**Polyaromatic hydrocarbon**	**-**	**Unintentionally formed during combustion**
2-OH-PH	2-Hydroxy-phenanthrene	605-55-0	PAH metabolite
1-HP	1-Hydroxy-pyrene	5315-79-7	PAH metabolite
Chlorpyrifos		**2921-88-2**	**Insecticide**
TCP	Trichloropyridinol	65156-38-4	Chlorpyrifos metabolite
Pyrethroids			**Insecticides**
3-PBA	3-Phenoxybenzoic acid	3739-38-6	Pyrethroid metabolite

Substance abbreviation	Substance full name	CAS #	Industrial use
Other substances			
TCS	Triclosan	3380-34-5	Biocide
BHA	3-Tert-butyl-4-hydroxyanisole	121-00-6	Antioxidant
BP3	Benzophenone-3	131-57-7	UV filter

Table S3. Summary of concentrations of substances measured in blood in RMA (N=1082).

Substances	Units	Quantified[a] Median (Range)	N <LOD or LOQ (LOD or LOQ concentration)	Determined[b] Median (Range)	N = 0[c]	N = Missing
Cr	µg/L whole blood	0.58 (<0.2, 2.95)	3 (0.2)	0.58 (0.18, 2.95)	0	0
Mn	µg/L whole blood	10.5 (<0.16, 32.6)	1 (0.16)	10.5 (0.01, 32.6)	0	0
Co	µg/L whole blood	0.18 (<0.05, 0.73)	22 (0.05)	0.18 (0, 0.73)	1	0
Ni	µg/L whole blood	0.62 (<0.21, 3.62)	1 (0.21)	0.62 (0, 3.62)	1	0
Se	µg/L whole blood	95.1 (<5, 198)	1 (5)	95.1 (0.11, 198)	0	0
Cd	µg/L whole blood	0.12 (<0.05, 3.9)	30 (0.05)	0.12 (1e-03, 3.9)	0	0
Hg	µg/L whole blood	0.72 (<0.05, 14.5)	8 (0.05)	0.72 (0, 14.5)	1	0
Pb	µg/L whole blood	7.14 (<0.07, 139)	1 (0.07)	7.14 (0.01, 139)	0	0
Al	µg/L whole blood	<5 (<5, 94.8)	571 (5)	4.79 (1e-04, 94.8)	0	6
PeCB	pg/mL serum	<10 (<10, 15.2)	1080 (10)	0.72 (0, 15.2)	192	0
HCB	pg/mL serum	42.5 (11.7, 7420)	0 (10)	42.5 (11.7, 7420)	0	0
alpha-HCH	pg/mL serum	<20 (<20, <20)	1082 (20)	0 (0, 17.6)	730	0
beta-HCH	pg/mL serum	<15 (<15, 528)	1025 (15)	2.98 (0, 528)	9	0
gamma-HCH	pg/mL serum	<20 (<20, 93.8)	1076 (20)	0 (0, 93.8)	707	0
Oxychlordane	pg/mL serum	<25 (<25, 29.2)	1080 (25)	0 (0, 29.2)	587	0
trans-Nonachlor	pg/mL serum	<5 (<5, 64)	836 (5)	2.84 (0.30, 64)	0	0
p,p'-DDT	pg/mL serum	<15 (<15, 404)	1047 (15)	0.37 (0, 404)	523	0
p,p'-DDE	pg/mL serum	92.7 (<40, 6130)	78 (40)	92.7 (9.8, 6130)	0	0
PCB-74	pg/mL serum	<5 (<5, 1050)	817 (5)	3.32 (0.43, 1050)	0	0
PCB-99	pg/mL serum	<5 (<5, 145)	733 (5)	3.91 (0.29, 145)	0	0
PCB-118	pg/mL serum	6.42 (<5, 94.5)	328 (5)	6.42 (0.73, 94.5)	0	0
PCB-138	pg/mL serum	26.7 (<5, 255)	9 (5)	26.7 (1.61, 255)	0	0
PCB-153	pg/mL serum	43.3 (<5, 298)	1 (5)	43.3 (4.4, 298)	0	0
PCB-156	pg/mL serum	<5 (<5, 65.1)	653 (5)	4.09 (0, 65.1)	1	0
PCB-170	pg/mL serum	11.9 (<5, 141)	178 (5)	11.9 (0.4, 141)	0	0
PCB-180	pg/mL serum	23.7 (<5, 402)	42 (5)	23.7 (1.55, 402)	0	0
PCB-183	pg/mL serum	<5 (<5, 46.7)	988 (5)	2.17 (0, 46.7)	1	0
PCB-187	pg/mL serum	5.49 (<5, 148)	497 (5)	5.49 (0.34, 148)	0	0
BDE-47	pg/mL serum	<15 (<15, 232)	1060 (15)	1.84 (0, 232)	137	0
BDE-99	pg/mL serum	<15 (<15, 170)	1074 (15)	0.87 (0, 170)	262	0
BDE-153	pg/mL serum	<15 (<15, 158)	1076 (15)	1.21 (0, 158)	87	0
L-PFOA	ng/g serum	1.2 (<LOQ, 9.75)	2 (0.02 – 0.29)	1.2 (<LOD, 9.75)	1	0
PFNA	ng/g serum	0.38 (<LOQ, 2.79)	78 (0.06 – 0.18)	0.38 (<LOD, 2.79)	53	0
PFDA	ng/g serum	<LOQ (<LOQ, 1.35)	409 (0.03 – 0.1)	0.16 (<LOD, 1.35)	216	0
PFUnDA	ng/g serum	<LOQ (<LOQ, 1.01)	574 (0.02 – 0.12)	<LOD (<LOD, 1.01)	332	0
L-PFHxS	ng/g serum	<LOQ (<LOQ, 255)	86 (0.02 – 0.22)	0.4 (<LOD, 255)	7	0
L-PFOS	ng/g serum	1.99 (<LOQ, 127)	0 (0.06 – 0.57)	1.99 (0.28, 127)	0	0
br-PFOS	ng/g serum	0.92 (<LOQ, 110)	22 (0.03 – 0.26)	0.92 (<LOD, 110)	0	0

[a] Concentrations below LOQ or LOD are reported as <LOQ or LOD (depending on the substance analysis method). PFAS LOQs and LODs varied between analytical batches and the highest LOQ/LOD for each substance was used in the summary statistics (see article section Handling of data below LOQ/LOD).

[b] Determined concentrations included concentrations below LOQ/LOD that were reported being above background noise (i.e. machine reads) as estimated in the analytical run. Concentrations were set to zero in cases when machine reads were below the background noise (see article section Handling of data below LOQ/LOD).

[c] Number of samples with concentrations set to 0 in the analyses.

Table S4. Summary of concentrations of substances measured in urine in RMA (N=1082).

Substances	Units	Quantified[a] Median (Range)	N < LOD (LOD concentration)	Determined[b] Median (Range)	N = 0[c]	N = Missing
MEP	ng/mL urine	32.9 (3.43, 8300)	0 (0.2)	32.9 (3.43, 8300)	0	0
MnBP	ng/mL urine	40.1 (3.76, 916)	0 (1.6)	40.1 (3.76, 916)	0	6
MBzP	ng/mL urine	6.47 (0.35, 231)	0 (0.3)	6.47 (0.35, 231)	0	0
MEHP	ng/mL urine	1.61 (<0.3, 290)	1 (0.3)	1.61 (0.27, 290)	0	0
5OH-MEHP	ng/mL urine	7.68 (<0.1, 1140)	2 (0.1)	7.68 (0.01, 1140)	0	0
5oxo-MEHP	ng/mL urine	6.02 (0.27, 1060)	0 (0.2)	6.02 (0.27, 1060)	0	0
5cx-MEPP	ng/mL urine	6.92 (0.86, 912)	0 (0.07)	6.92 (0.86, 912)	0	0
2cx-MEHP	ng/mL urine	2.03 (0.21, 90.9)	0 (0.05)	2.03 (0.21, 90.9)	0	0
OH-MiNP	ng/mL urine	3.7 (0.46, 635)	0 (0.05)	3.7 (0.46, 635)	0	0
oxo-MiNP	ng/mL urine	1.81 (0.09, 228)	0 (0.05)	1.81 (0.09, 228)	0	0
cx-MiNP	ng/mL urine	5.87 (0.55, 1130)	0 (0.05)	5.87 (0.55, 1130)	0	0
cx-MiDP	ng/mL urine	0.39 (<0.1, 20)	36 (0.1)	0.39 (0.03, 20)	0	0
OH-MPHP	ng/mL urine	1.01 (<0.08, 92.1)	1 (0.08)	1.01 (0.07, 92.1)	0	0
oxo-MiNCH	ng/mL urine	1.01 (<0.08, 1100)	1 (0.08)	1.01 (0.03, 1100)	0	0
cx-MINCH	ng/mL urine	0.78 (<0.1, 391)	10 (0.1)	0.79 (0, 391)	1	7
OH-MINCH	ng/mL urine	0.76 (<0.1, 672)	4 (0.1)	0.76 (0.05, 672)	0	7
DPP	ng/mL urine	1.89 (0.29, 45.4)	0 (0.07)	1.89 (0.29, 45.4)	0	3
DBP	ng/mL urine	0.15 (<0.05, 11.7)	116 (0.05)	0.15 (0, 11.7)	1	3
BBOEP	ng/mL urine	<0.05 (<0.05, 2.14)	564 (0.05)	0.04 (1e-03, 2.14)	0	3
BPA	ng/mL urine	0.9 (<0.2, 49.4)	48 (0.2)	0.9 (0, 49.4)	5	0
BPS	ng/mL urine	0.13 (<0.03, 49.4)	33 (0.03)	0.13 (2e-03, 49.4)	0	1
4,4BPF	ng/mL urine	0.1 (<0.03, 207)	204 (0.03)	0.1 (0, 207)	22	0
2-OH-PH	ng/mL urine	0.15 (<0.1, 7.09)	308 (0.1)	0.15 (0.01, 7.09)	0	0
1-HP	ng/mL urine	<0.1 (<0.1, 4.12)	692 (0.1)	0.08 (0, 4.12)	14	0
TCP	ng/mL urine	1.19 (0.15, 50.6)	0 (0.07)	1.19 (0.15, 50.6)	0	2
3-PBA	ng/mL urine	0.26 (<0.05, 11.7)	12 (0.05)	0.26 (0.01, 11.7)	0	1
TCS	ng/mL urine	0.28 (<0.1, 1130)	181 (0.1)	0.28 (0, 1130)	6	0
BHA	ng/mL urine	0.9 (<0.02, 480)	137(0.02)	0.9 (0, 480)	28	1
BP3	ng/mL urine	0.76 (<0.2, 1250)	145 (0.2)	0.76 (0.01, 1250)	0	1

[a] Concentrations below LOD are reported as <LOD.
[b] Determined concentrations included concentrations below LOD that were reported being above background noise (i.e. machine reads) as estimated in the analytical run. Concentrations were set to zero in cases when machine reads were below the background noise (see article section Handling of data below LOD).
[b] Number of samples with concentrations set to 0 in the analyses.

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Full length article

Socio-demographic inequalities influence differences in the chemical exposome among Swedish adolescents

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ABSTRACT

Relatively little is known about the relationship between socio-demographic factors and the chemical exposome in adolescent populations. This knowledge gap hampers global efforts to meet certain UN sustainability goals. The present work addresses this problem in Swedish adolescents by discerning patterns within the chemical exposome and identify demographic groups susceptible to heightened exposures.

Enlisting the Riksmaten Adolescents 2016–17 (RMA) study population (N = 1082) in human-biomonitoring, and using proportional odds ordinal logistic regression models, we examined the associations between concentrations of a diverse array of substances (N = 63) with the determinants: gender, age, participant/maternal birth country income per capita level, parental education levels, and geographic place of living (longitude/latitude).

Participant/maternal birth country exhibited a significant association with the concentrations of 46 substances, followed by gender (N = 41), and longitude (N = 37). Notably, individuals born in high-income countries by high-income country mothers demonstrated substantially higher estimated adjusted means (EAM) concentrations of polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and per- and polyfluoroalkyl substances (PFASs) compared to those born in low-income countries by low-income country mothers. A reverse trend was observed for cobalt (Co), cadmium (Cd), lead (Pb), aluminium (Al), chlorinated pesticides, and phthalate metabolites. Males exhibited higher EAM concentrations of chromium (Cr), mercury (Hg), Pb, PCBs, chlorinated pesticides, BFRs and PFASs than females. In contrast, females displayed higher EAM concentrations of Mn, Co, Cd and metabolites of phthalates and phosphorous flame retardants, and phenolic substances. Geographical disparities, indicative of north-to-south or west-to-east substance concentrations gradients, were identified in Sweden. Only a limited number of lifestyle, physiological and dietary factors were identified as possible drivers of demographic inequalities for specific substances.

This research underscores birth country, gender, and geographical disparities as contributors to exposure differences among Swedish adolescents. Identifying underlying drivers is crucial to addressing societal inequalities associated with chemical exposure and aligning with UN sustainability goals.

1. Introduction

In 2015, the United Nations (UN) established a set of 17 sustainable development goals to be achieved by 2030 (U.N., 2015). Several of these goals are particularly relevant to the issue of chemical pollution, notably Goal 3: "Ensure healthy lives and promote well-being", Goal 5: "Gender equality" and Goal 10: "Reduce inequality within and among countries". According to the Lancet Commission (Collaborators, 2017), the

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Table 1
Demographic characteristics of the participants in Riksmaten Adolescents 2016–2017.

	5th Grade (N = 329)	8th Grade (N = 405)	11th Grade (N = 348)	Total (N = 1082)
Gender				
Female	164 (49.8 %)	226 (55.8 %)	216 (62.1 %)	606 (56.0 %)
Male	165 (50.2 %)	179 (44.2 %)	132 (37.9 %)	476 (44.0 %)
Age (years)				
Mean ± (SD)	11.6 (0.4)	14.5 (0.4)	17.8 (0.7)	14.7 (2.5)
Median	11.5	14.5	17.7	14.6
Range	10.6–13.1	11.6–15.7	16.8–21.1	10.6–21.1
Participant | Maternal birth country per capita income				
N-missing	4	2	0	6
Low | Low	15 (4.6 %)	10 (2.5 %)	20 (5.7 %)	45 (4.2 %)
High | Low	7 (2.2 %)	15 (3.7 %)	4 (1.1 %)	26 (2.4 %)
Upper-middle |Upper-middle	6 (1.8 %)	10 (2.5 %)	21 (6.0 %)	37 (3.4 %)
High | Upper-middle	37 (11.4 %)	25 (6.2 %)	15 (4.3 %)	77 (7.2 %)
High | High	260 (80.0 %)	343 (85.1 %)	288 (82.8 %)	891 (82.8 %)
Maternal education level				
N-missing	13	20	25	58
Elementary or None	27 (8.5 %)	28 (7.3 %)	27 (8.4 %)	82 (8.0 %)
≤2 years Secondary	43 (13.6 %)	49 (12.7 %)	69 (21.4 %)	161 (15.7 %)
≥3 years Secondary	79 (25.0 %)	77 (20.0 %)	71 (22.0 %)	227 (22.2 %)
Higher Education	167 (52.8 %)	231 (60.0 %)	156 (48.3 %)	554 (54.1 %)
Paternal education level				
N-missing	17	26	38	81
Elementary or None	29 (9.3 %)	39 (10.3 %)	41 (13.2 %)	109 (10.9 %)
≤ 2 years Secondary	59 (18.9 %)	72 (19.0 %)	82 (26.5 %)	213 (21.3 %)
≥ 3 years Secondary	105 (33.7 %)	87 (23.0 %)	85 (27.4 %)	277 (27.7 %)
Higher Education	119 (38.1 %)	181 (47.8 %)	102 (32.9 %)	402 (40.2 %)

sustainable global management and reduction of chemicals is a critical factor in attaining Goal 3. Indirect benefits of pollution mitigation include enhancing gender equity (Goal 5) (Collaborators, 2017), due to gender-related differences in lifestyle that affect chemical exposure and biological disparities in chemical toxico-kinetics and toxico-dynamics (Liang et al., 2018; Sakali et al., 2021). Furthermore, regional and country disparities in the development of industrial and agricultural/forestry sectors contribute to inequalities in chemical pollution exposure (Berg et al., 2017). To address these challenges, it is imperative to gain a better understanding of socio-demographic differences in the body burdens of chemicals in humans. Such knowledge is essential for identifying populations at risk of high chemical exposure, ultimately improving our capacity to manage chemicals effectively and support sustainable global development.

Regrettably, there is a lack of population-based studies that have examined the connections between socio-demographic factors and exposure to a diverse spectrum of toxic substances in adolescents. Although there has been an uptick in public health attention and scientific interest in understanding the exposome at large, that being all internal and external stressors on the body, little is still known specifically about the chemical component of the exposome (i.e., the 'chemical exposome'). Previous investigations have predominantly focused on associations with individual chemical compounds or chemical categories. In contrast to adulthood, adolescence may represent a critical period of heightened vulnerability to the adverse effects of chemical exposure, primarily due to the profound physiological changes that transpire during this developmental phase (Abreu and Kaiser, 2016). Adolescence is also characterised by rapid and substantial shifts in behaviour, which may exert a pronounced influence on patterns of chemical exposure. Recent national and regional, population-based studies on adolescents in Germany (GerES V 2014–17), the United States (NHANES, 2011–16) and Belgium (FLEHS IV 2016–2020) have published data pertaining to the body burden of numerous chemical groups measured in whole blood, serum and urine (Bandow et al., 2020; Fillol et al., 2021; Murawski et al., 2020; NHANES, 2022; Schoeters et al., 2022; Schulz et al., 2021; Schwedler et al., 2020b; Tschersich et al., 2021). Some of these investigations have reported chemical body burdens in relation to age, sex, socioeconomic status, and immigration status. However, to the best of our knowledge, only the FLEHS IV study has disseminated its findings in a single publication, employing a more holistic approach in exploring the interplay between gender, age, and sociodemographic variables in relation to the chemical exposome (Schoeters et al., 2022). Moreover, studies investigating the association between country of birth and chemical body burdens in adolescents have remained notably scarce.

In Sweden, the recent nation-wide survey of adolescents, Riksmaten Adolescents 2016–17 (RMA), showed that Swedish adolescents were exposed to a variety of toxic substances, including elements, halogenated persistent organic pollutants (POPs), phthalates, phosphorous flame retardants (PFRs), pesticides and bisphenols and other potentially toxic phenolic substances (Pineda et al., 2023). Parts of the study population had concentrations of some elements, POPs and pesticides that exceeded the most conservative human biomonitoring guidance values (HBM-GVs) or other proposed health-based guidance values (Pineda et al., 2023).

In a recently published paper based on the RMA study, concentrations of per- and polyfluoroalkyl substances (PFASs) in serum were strongly associated with gender and immigration status, but less so with age and maternal/paternal education level (Nyström et al., 2022). In the present study we expanded the previous investigations on RMA to explore potential socio-demographic disparities in body burdens of elements, chlorinated, brominated and fluorinated persistent organic pollutants (POPs), phthalates, phthalate alternatives, phosphorous flame retardants, bisphenols, and other phenolic substances among the RMA participants, by determining the relationship between measured substance concentrations with age, gender, birth country income per capita, parental education levels, and latitude/longitude of residence in Sweden. Furthermore, in instances where significant associations emerged with demographic factors, we investigated if some previously reported determinants of body burdens of the studied substances in Sweden, such as diet, smoking, and body mass index (BMI), could at least partially explain some of the observed demography-related associations.

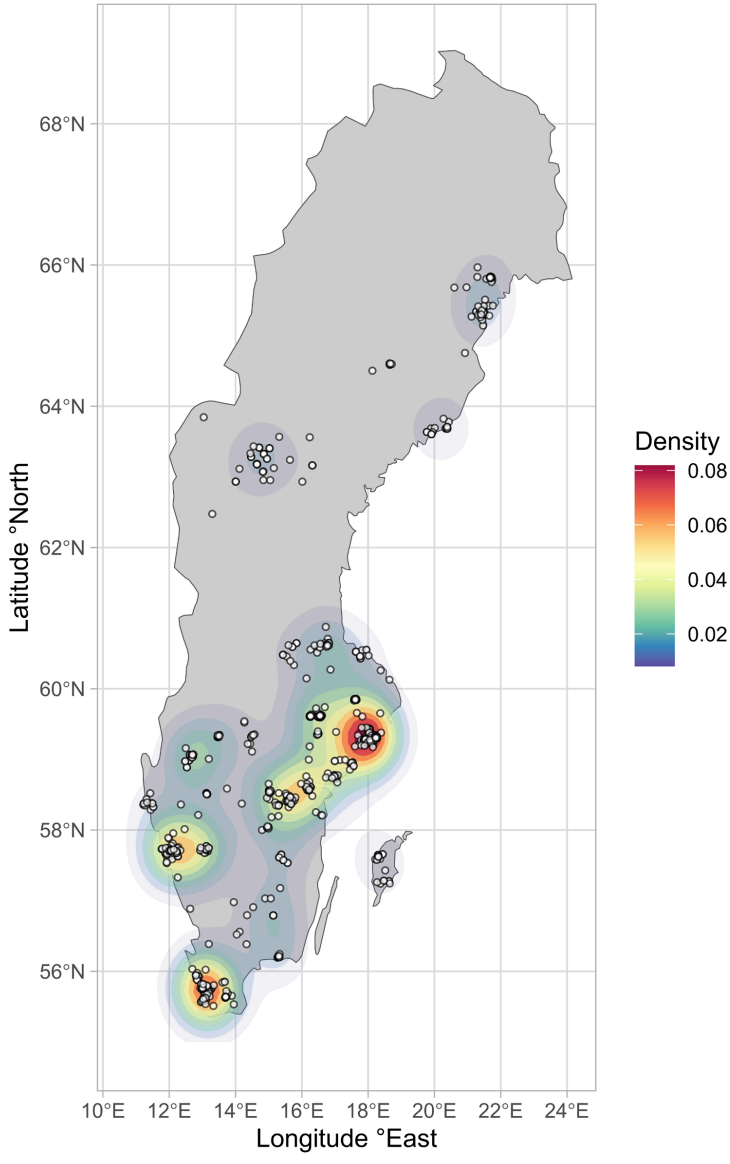

Fig. 1. Map of Sweden demonstrating the coordinates of all 1082 participants zip code of home address (white dots) included in this study. Coloured contours represent the spatial density relative to the study populations coordinates. Sweden encompasses latitudes ~ 55° to ~ 69° north, and longitudes ~ 11° to ~ 24° east, with the largest urban areas in the south-west being Malmö (55.6°N, 13°E) at the south Baltic Sea coast, and Gothenburg (57.7°N, 11.9°E) at the Atlantic Ocean coast, along with Stockholm (59.3°N, 18°E) in the east at the central Baltic Sea coast.

2. Methods

2.1. Study group and design

The study population was a sub-sample of RMA 2016–17, a nationally representative cross-sectional school-based dietary survey of Swedish adolescents in school grades 5 (average 12-year-olds), 8 (15-year-olds) and 11 (18-year-olds) conducted by the Swedish Food Agency (Table 1 and Fig. 1). Details about the recruitment process and study design are described in Pineda et al. (2023) and Moraeus et al. (2018). In short, a web-based dietary assessment method (RiksmatenFlexDiet), and four online-based questionnaires (RiksmatenFlexQ) were used to collect

Table 2
Concentrations of substances included in this study (N = 1082). Substances concentrations in urine adjusted for density.

Substances[1]	Median (Range)[2]	N < LOQ or LOD	N = 0[3]
Chromium (Cr)	0.58 (<0.2, 2.95)	3	0
Manganese (Mn)	10.5 (<0.16, 32.6)	1	0
Cobalt (Co)	0.12 (<0.05, 0.73)	22	1
Nickel (Ni)	0.62 (<0.21, 3.62)	1	1
Selenium (Se)	95.1 (<5, 198)	1	0
Cadmium (Cd)	0.12 (<0.05, 3.9)	30	0
Mercury (Hg)	0.72 (<0.05, 14.5)	8	1
Lead (Pb)	7.15 (<0.07, 139)	1	0
Aluminium (Al)	<5 (<5, 94.8)	571	0
Pentachlorobenzene (PeCB)	<10 (<10, 15.2)	1080	192
Hexachlorbenzene (HCB)	42.5 (11.7, 7420)	0	0
alpha-Hexachlorocyclohexane (α–HCH)	<20 (<20, <20)	1082	731
beta-Hexachlorocyclohexane (β–HCH)	<15 (<15, 528)	1025	9
gamma-Hexachlorocyclohexane (γ–HCH)	<20 (<20, 93,8)	1077	708
Oxychlordane	<25 (<25, 29.2)	1081	588
trans-Nonachlor	<5 (<5, 64)	836	0
1,1-bis-(4-chlorophenyl)-2,2,2-trichlorethane (p,p'-DDT)	<15 (<15, 404)	1048	524
1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE)	92.6 (<40, 6130)	78	0
2,4,4′,5-Tetrachlorobiphenyl (PCB-74)	<5 (<5, 1050)	817	0
2,2′,4,4′,5-Pentachlorobiphenyl (PCB-99)	<5 (<5, 145)	733	0
2,3′,4,4′,5-Pentachlorobiphenyl (PCB-118)	6.43 (<5, 94.5)	328	0
2,2′,3,4,4′,5′-Hexachlorobiphenyl (PCB-138)	26.7 (<5, 255)	9	0
2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB-153)	43.3 (<5, 298)	1	0
2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB-156)	<5 (<5, 65.1)	653	1
2,2′,3,3′,4,4′,5-Heptachlorobiphenyl (PCB-170)	11.9 (<5, 141)	178	0
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl (PCB-180)	23.7 (<5, 402)	42	0
2,2′,3,4,4′,5′,6-Heptachlorobiphenyl (PCB-183)	<5 (<5, 46.7)	988	1
2,2′,3,4′,5,5′,6-Heptachlorobiphenyl (PCB-187)	5.48 (<5, 148)	497	0
2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47)	<15 (<15, 232)	1060	137
2,2′,4,4′,5-Pentabromodiphenyl ether (BDE-99)	<15 (<15, 170)	1074	262
2,2′,4,4′,5,5′-Hexabromodiphenyl ether (BDE-153)	<15 (<15, 158)	1076	87
Linear-Perfluorooctanoic acid (L-PFOA)	1.2 (<LOQ, 9.75)	2	1
Perfluorononanoic acid (PFNA)	0.38 (<LOQ, 2.79)	78	53
Perfluorodecanoic acid (PFDA)	<LOQ (<LOQ, 1.35)	409	216
Perfluoroundecanoic acid (PFUnDA)	<LOQ (<LOQ, 1.01)	574	332
Linear Perfluorohexanesulfonic acid (L-PFHxS)	<LOQ (<LOQ, 255)	86	7
Linear Perfluorooctanesulfonic acid (L-PFOS)	1.99 (<LOQ, 127)	0	0
Branched Perfluorooctanesulfonic acid (br-PFOS)	0.92 (<LOQ, 110)	22	0
Monoethyl phthalate (MEP)	32.8 (3.43, 8300)	0	0
Mono-butyl phthalate (MBP)	40.1 (3.76, 916)	0	0
Monobenzyl phthalate (MBzP)	6.48 (0.35, 231)	0	0
Mono-(2-ethylhexyl) phthalate (MEHP)	1.61 (<0.3, 290)	1	0
Mono-(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP)	7.69 (<0.1, 1140)	2	0
Mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP)	6.01 (0.27, 1060)	0	0
Mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP)	6.91 (0.86, 912)	0	0
Mono(2-(carboxymethyl)hexyl) phthalate (2cx-MEHP)	2.03 (0.21, 90.9)	0	0
Mono-(4-methyl-7-hydroxyoctyl) phthalate (OH-MiNP)	3.68 (0.46, 635)	0	0
Mono-(4-methyl-7-oxooctyl) phthalate (oxo-MiNP)	1.8 (0.09, 228)	0	0
Mono-(4-methyl-7-carboxyheptyl) phthalate (cx-MiNP)	5.85 (0.55, 1130)	0	0
Monocarboxyisononyl phthalate (cx-MiDP)	0.4 (<0.1, 20)	36	0
6-Hydroxy monopropylheptyl phthalate (OH-MPHP)	1.01 (<0.08, 92.1)	1	0
Cyclohexane-1,2-dicarboxylate-mono(oxo-isononyl) ester (oxo-MINCH)	1.01 (<0.08, 1100)	1	0
1,2-Cyclohexanedicarboxylic Acid Mono 4-Methyl-7-carboxy-heptyl Ester (cx-MINCH)	0.79 (<0.1, 391)	10	1
2-(((Hydroxy-4-methyloctyl)oxy)carbonyl)cyclohexanecarboxylic acid (OH-MINCH)	0.76 (<0.1, 672)	4	0
Diphenyl phosphate (DPP)	1.89 (0.29, 45.4)	0	0
Dibutyl phosphate (DBP)	0.15 (<0.05, 11.7)	116	1
Bis(2-butoxyethyl) phosphate (BBOEP)	<0.05 (<0.05, 2.14)	564	0
Bisphenol A (BPA)	0.9 (<0.2, 49.4)	48	5
Bisphenol S (BPS)	0.13 (<0.03, 49.4)	33	0
4,4-Bisphenol F (4,4BPF)	0.1 (<0.03, 207)	204	22
2-hydroxy-phenanthrene (2-OH-PH)	0.15 (<0.1, 7.09)	308	0
1-Hydroxypyrene (1-HP)	<0.1 (<0.1, 4.12)	692	14
Trichloropyridinol (TCP)	1.19 (0.15, 50.6)	0	0
3-Phenoxybenzoic acid (3-PBA)	0.26 (<0.05, 11.7)	12	0
Triclosan (TCS)	0.28 (<0.1, 1130)	181	6
3-*tert*-Butyl-4-hydroxyanisole (BHA)	0.9 (<0.02, 494)	137	28
Benzophenone-3 (BP3)	0.76 (<0.2, 1250)	145	0

[1] Elements: μg/L whole blood, except Aluminium: μg/L serum; Chlorinated and brominated substances: pg/ml serum; Fluorinated substances: ng/g serum; Urine substances (phthalate metabolites, etc.): μg/L urine (density adjusted).

[2] LODs given for elements and substances measured in urine. LOQs given for chlorinated pesticides, PCBs, BFRs and PFASs. PFASs have range of LOQs depending on analysis batch; L-PFOA: 0.02–0.288, PFNA: 0.058–0.288, PFDA: 0.058–0.288, PFUnDA: 0.058–0.288, L-PFHxS: 0.022–0.463, L-PFOS: 0.056–0.562, br-PFOS: 0.056–0.562.

[3] Statistical analyses included concentrations below LOQ/LOD that were reported being above background noise (i.e. machine reads) as estimated in the analytical run. Concentrations were set to 0.0001 in cases when machine reads were below the background noise (see article section 2.5 Substance concentrations). N = 0: number of samples with concentrations below the background noise in the analyses.

data about demographic factors, lifestyle habits and food consumption (Moraeus et al., 2018; Nyström-Kandola et al., 2023). Participants retrospectively registered their food intake (24-h recall) on 2 non-consecutive days and filled in the questionnaires at home with the aid of parents if required (Moraeus et al., 2018). Of the 1305 students who accepted to donate blood and urine, 1111 participants had all three biomonitoring matrices (i.e., whole blood, blood serum and urine) analysed for substance concentrations. Of these participants, 28 participants were manually removed as large sections of data were incomplete, resulting in 1083 participants. Due to an inability to impute missing substance concentrations for a single participant (see article section 2.8 Imputation), the final number of participants included in the analysis became 1082.

Ethical approval was obtained from the Regional Ethical Review Board in Uppsala (No 2015/190). Written informed consent was obtained from all participants and from their legal guardians if younger than 16 years.

2.2. Sample collection

Non-fasting serum, plasma and whole blood samples were collected by trained staff from the regional divisions of Occupational and Environmental Medicine in Sweden during school visits as previously described in Pineda et al. (2023). Single-spot urine samples were collected by the individual participants during the same school visits. All samples were frozen at the sampling site and stored at −20 °C before transportation to the Swedish Food Agency, where they were stored at −80 °C before analysis.

2.3. Sample analysis

All of the analysed substances included in this study, along with full names and abbreviations are given in Table 2. Details on the analytical methods are provided in Pineda et al. (2023). Briefly, elements in whole blood and serum were analysed by the Department of Laboratory Medicine, Lund University, Lund, Sweden, using inductively coupled plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientific, Bremen, GmbH). Polychlorinated biphenyls (PCBs), chlorinated pesticides/metabolites and polybrominated diphenyl ethers (PBDEs) in serum (Table 2) were analysed at the Department of Health Security, National Institute for Health and Welfare, Kuopio, Finland, using gas chromatography - triple quadrupole mass spectrometry (GC–MS/MS, Agilent 7010 GC–MS/MS system, Wilmington, DE, U.S.). Per- and polyfluoroalkyl substances (PFASs) in serum (Table 2) were analysed by the Department of Environmental Science, Stockholm University, Stockholm, Sweden, using a Waters ACQUITY ultra performance liquid chromatograph (UPLC) coupled to a Waters Xevo TQS triple quadrupole mass spectrometer.

In urine, utilising a Shimadzu UFLC system (Shimadzu Corporation, Kyoto, Japan) coupled to a QTRAP5500 triple quadrupole linear ion trap mass spectrometer (LC-MS/MS; AB Sciex, Foster City, CA, U.S.), metabolites of phthalates, phthalate alternatives, phosphorous flame retardants (PFRs), pesticides and polycyclic aromatic hydrocarbons (PAHs) were measured (Table 2). In addition, the plastics chemicals bisphenols, the biocide triclosan (TCS), the preservative/antioxidant 3-*tert*-butyl-4-hydroxyanisole (BHA), and the UV filter benzophenone-3 (BP-3) were analysed (Table 2). Analyses were performed at the Department of Laboratory Medicine, Lund University, Lund, Sweden.

Ferritin was analysed in plasma samples by the accredited laboratory of the Department of Clinical Chemistry and Pharmacology, University Hospital in Uppsala, Sweden, using Abbott Architect ci8200 analysers (Abbott Laboratories, Abbott Park, IL, USA).

2.4. Data processing

All data processing, statistical analyses and figures were produced using R version 4.0.2 (22–06-2020). The cut-off significance level in the statistical analyses was set to $p \leq 0.05$.

2.5. Substance concentrations

As described in Pineda et al. (2023), measurable concentrations which were below limits of quantification or detection (LOQ and LOD, respectively), were used when reported by the laboratories to keep as low of a bias as possible within the statistical analyses (AMC, 2001; Bergstrand and Karlsson, 2009). However, when interpreting the results, it is important to consider that measured concentrations below LOD or LOQ are more uncertain than concentrations above these limits. Concentrations reported by the laboratories to be at or below 0 were converted to 0.0001 independent of unit of concentration, a number selected as it is lower than any value reported by the laboratories. This value would represent the assumption that although a substance had been reported at or below 0 within a sample, it is highly unlikely that there was absolutely no trace of the substance at all in the sample. For the PFASs, concentrations < LOD were deemed too uncertain by the analytical laboratory, thus in the statistical analyses PFASs concentrations < LOD were set to 0.0001. Only substances with < 30 % of the concentrations set to 0.0001 (Table 2), and/or with models demonstrating goodness of fit (Fig. S.1) were included in the final statistical analyses.

2.6. Demographic determinants

Characteristics of the socio-demographics of the studied population are presented in Table 1. The 'Participant | Maternal birth country income per capita level' variable was combined from two individual variables: 'Participant birth-country income per capita level' and 'Maternal birth-country income per capita level'. The combination of the participant/maternal birth country was done to avoid multicollinearity bias within the model as the variables were highly correlated to each other. The reasoning behind including maternal birth country in the exposome analyses was that exposure to some chlorinated POPs early in foetal life and during breast-feeding, are detectable in humans for decades after cessation of these exposures (Glynn et al., 2007; Lignell et al., 2011; Wesselink et al., 2019). Moreover, after the move to Sweden there may be birth country differences in sources of exposure affected by housing conditions, and the participant use of products and dietary habits. A maternal influence on lifestyle and diet of the adolescents is also possible, thus potentially affecting more recent exposure to the toxic substances in Sweden. Birth country reported by the RMA participants and their biological mothers was classified according to the World Bank Country Classification (WBG, 2018), based on each countrýs gross national income per capita. The combined participant/maternal birth country variable was primary ordered based on the maternal birth country in the order; low, lower-middle, upper-middle, high-income country. Secondary the variable was ordered according to the participants birth country generating the order for participant/maternal birth country; 'Low | Low', 'High | Low', 'Upper-middle | Upper-middle',

'High | Upper-middle, 'High | High' birth country (Table 1). Maternal and Paternal Education variables were both ordered as: Elementary school or none (ES), < 2 years of upper-secondary or vocational (2-Us), 3 Years or more of upper-secondary or vocational (3 + Us), and Higher education (HE) (Table 2). Latitude and longitude coordinates were derived from the ZIP codes of the participants home address using the Google Maps Platform API. In the case of 20 participants which had missing ZIP codes, the latitude and longitude coordinates of their corresponding school address were used instead as an approximation. Spearman's rank correlation coefficients were calculated for all demographical determinants in the base model except for gender due it being a dichotomous variable with no inherent order (Fig. S.3).

2.7. Additional determinants in secondary models

Some additional determinants were explored as possible explanatory determinants of observed demographic differences in substance concentrations. These determinants were chosen based on statistically significant relationships with body burdens of the studied substances reported from other studies of Swedish populations, or in some cases in other populations, and the availability of data in RMA (Table S.1). For example, BMI, smoking, consumption of alcohol, eggs and seafood have been shown to be significantly related to PCBs body burdens in different populations in Sweden and were therefore added as additional determinants in the PCBs regression models (Table S.1 and S.40-S.77). Season of sampling was included in all secondary models, since previous studies have reported seasonal variation of blood levels of some of the elements, POPs, and substances in urine included in this study (Table S.1) (Bastiaensen et al., 2021a; Geller et al., 2023; Koppen et al., 2009; Makey et al., 2014).

It was assumed that all 5th graders were non-smokers and non-consumers of alcohol, as no data were collected from these participants. For other participants, the variable 'current smoking habits' was ordered as follows: 'No', 'Quit', 'Yes'. For 'alcohol consumption the last 6 months', the variable was ordered as: 'No', 'Yes, once', 'Yes, several times'. Food consumption variables (Table S.1); game meat products, chocolate, ice cream and tube packaged foods were based on participant answers given for frequency questions about consumption the prior year and were recoded, condensed and ordered as follows: 'Never' = 'Never', '< 1 per month' = 'Seldom', 'Once per month' = 'Irregularly monthly', '2–3 times per month' = 'Often monthly', 'Once per week' = 'Irregularly weekly, '2–3 times a week' = 'Often weekly', '4–6 times a week' = 'Often weekly', 'Every day' = 'Often weekly'. Canned fish were summed from three separate yearly consumption variables; canned herring/mackerel, canned tuna, and canned anchovies/sardines, where their original frequencies were translated to numeric integers as follows: 'Never' = 0, '1–3 times a year' = 2, '4–8 times a year' = 6, '9–11 times a year' = 24, 'Once per week' = 52, 'Twice a week' = 104, '×3 times a week' = 156, 'Once a day or more' = 365.

To estimate consumption of seafood, meat and eggs (g/day) over a longer time period (Table S.1), data from the two days of 24-h dietary recall were transformed to habitual (long-term) intake using the Multiple Source Method (Harttig et al., 2011). These calculations were based on the total participating population of the RMA study (Nyström-Kandola et al., 2023). Habitual egg consumption was calculated with the assumption that all individuals consumed it to some extent, therefore meaning that all individuals were regarded as consumers. This was not the case for habitual meat and seafood consumption where individuals could be identified and represented as non-consumers. BMI classification was based on the IOTF cut-offs dependent on age and sex for those under 18 (Cole and Lobstein, 2012). For participants 18 and older, standard IOTF BMI definitions for adults were applied (Cole and Lobstein, 2012). BMI was divided into four groups: Underweight, Normal weight, Overweight and Obese (Table S.1).

2.8. Imputation

From the remaining data set of 1083 participants, there were 605 participants with complete data across all variables, with the remaining 478 participants having at least 1 or more missing values in any of the dependent or explanatory variables (Table 1). Among the substances analysed; L-PFOA, BPS, 3-PBA, BHA and BP3 had 1 missing value each; TCP 2 values; DPP, DBP and BBOEP 3 values each; Al and MBP 6 values each; cx-MiNCH and OH-MiNCH 7 values each. Missing values were imputed using MICE package version 3.10.0 for R (13–07-2020) using Rubin's rules across 16 multiple imputations and 5 iterations. All missing values were successfully imputed apart from a single participant who was manually removed from the analysis with a missing value of L-PFOA which could not be successfully imputed from the other variables, bringing the final number of included participants to 1082.

2.9. Ordinal regression models

All associations between substance concentrations and possible determinants were analysed using Ordinal Regression Models (ORM), also known as 'proportional odds ordinal logistic regression'. Bias and increased variation due to the multiple imputation were accounted for within the models by using the 'fit.mult.impute' function from the Hmisc package (ver. 4.4–0) with an ORM fitter from the 'rms' package (ver. 6.0–0) for R. Additionally, the model fits were adjusted for clustering of the schools using the 'robcov' function from the Hmisc package utilising the Huber-White method to adjust the variance–covariance matrix correctly for heteroscedasticity. The same base model was applied to all contaminants which included the covariates; age, gender, participant/maternal birth country income per capita, maternal and paternal education, latitude, longitude and the combined effect of latitude * longitude. This can be expressed in the formal equation:

$$logit(\Pr(y \leq C_k|X)) = a_k - X_b$$

Where $X_b = b_{Age} + b_{Gender} + b_{Participant/maternal\ birth\ country\ income\ per\ capita} + b_{Maternal\ education} + b_{Paternal\ education} + b_{Latitude} + b_{Longitude} + b_{(Latitude\ *\ Longitude)}$ and C_k is the k^{th} ordered outcome value.

Summary tables for each substance base model were created demonstrating the odds ratios for each variable as well as ANOVA tables calculated for each substance model providing the chi-square, degrees of freedom, and p-value for each variable (see Supplement, Tables S.2 – S.167). Estimated adjusted means (EAM) of substance concentrations for each determinant were computed from the ordinal regression models using the smearing estimation nonparametric method (Duan, 1983). Values from the ANOVA result tables were used to create a p-value heatmap and chi-square proportion heat map (Figs. 2 and S.2). In the chi-square heatmap (Fig. S.2) each covariates contribution to the variation of substance concentrations in each separate model was evaluated by dividing the chi-square of each variable by the total sum of all chi-squares for any given model.

3. Results and Discussion

In our study on the associations between concentrations of 63 toxic substances and demographic determinants in Swedish adolescents, the ORMs uniformly incorporated seven demographic determinants for all substances. As a result, any statistically significant relationship between a determinant and substance concentrations has been adjusted for the effects of the remaining six determinants.

Spearman's rank correlation coefficients showed that most social-demographic determinants were not correlated to each other with some minor exceptions (Fig. S.3). Maternal and paternal education levels were as expected significantly and moderately correlated (r = 0.46) to each other, and so was latitude to longitude (r = 0.62) (Fig. S.3). The positive correlation between latitude and longitude was likely a

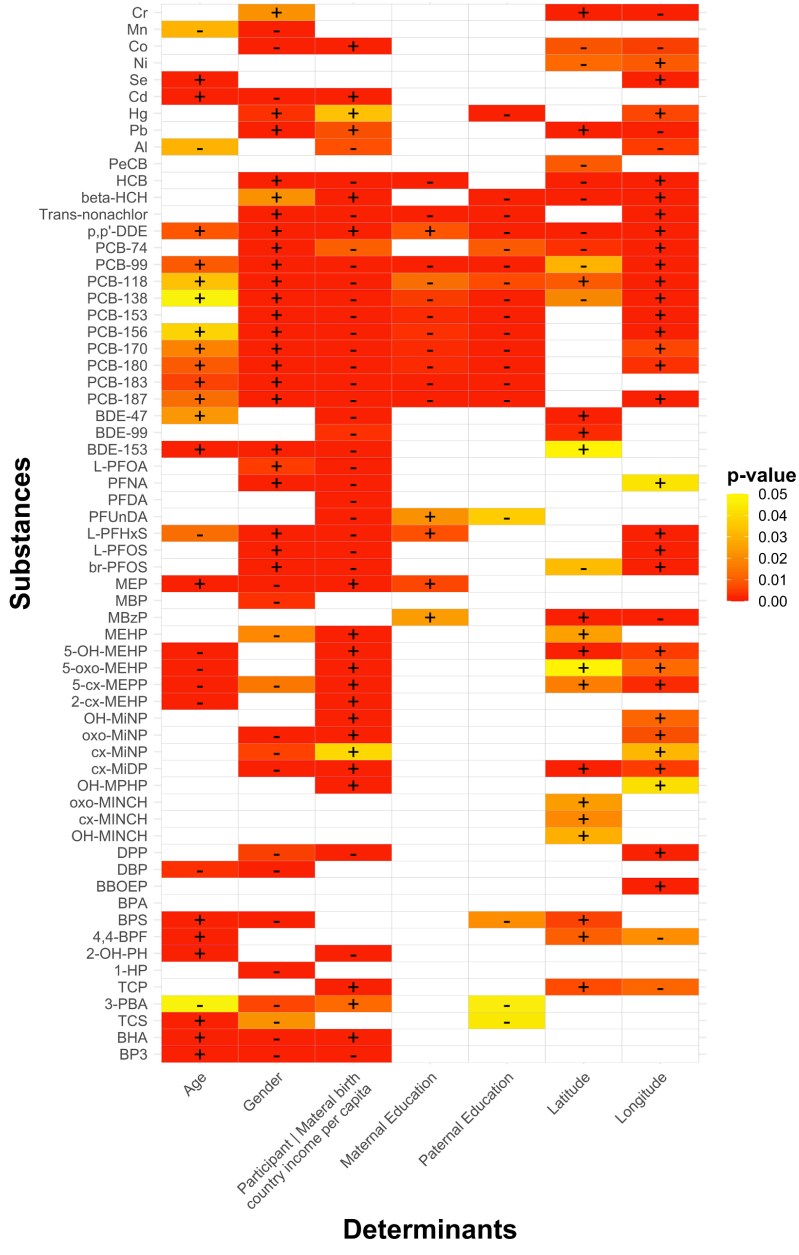

Fig. 2. Heat map demonstrating an overview of p-values for associations between substance concentrations and the demographic determinants obtained from the base ordinal regression models. The symbol within each cell indicates the direction of the association; if positive (+) with odds-ratio > 1 or negative (-) with odds-ratio < 1 (Tables S.2 - S.167). In the case of categorical variables, (i.e., gender, P|M birth country and parental education) the association direction is calculated from the odds-ratio comparing the lowest level (low|low per capita income; elementary school or none) with the highest level (high|high, higher education) or with female as the reference category (See 'Demographic determinants').

consequence of having two major metropolitan areas in Sweden (i.e., Malmö/Gothenburg) located in the south-west of the country whilst the other major population centres (e.g., Stockholm and Umeå) are generally located towards the east coast as you progress north through the country, as indicated by the distribution of the participants (Fig. 1). Participant/maternal birth country income per capita was weakly correlated with maternal education (r = 0.22) (Fig. S.3).

Participant/maternal birth country income per capita had the largest number of significant associations across all substances (N = 46), followed by gender (N = 41), longitude (N = 37), age (N = 29), latitude (N = 28), paternal education (N = 18), and maternal education (N = 16) (Fig. 2). Participant/maternal birth country income per capita in many cases tended to explain the largest part of the variation in substance concentrations compared to other covariates (Fig. S.2), suggesting that there are birth country-related inequities in exposures of Swedish adolescents.

The elements had varied combinations of significant determinants (Fig. 2). This is in concordance with the generally negligible-weak

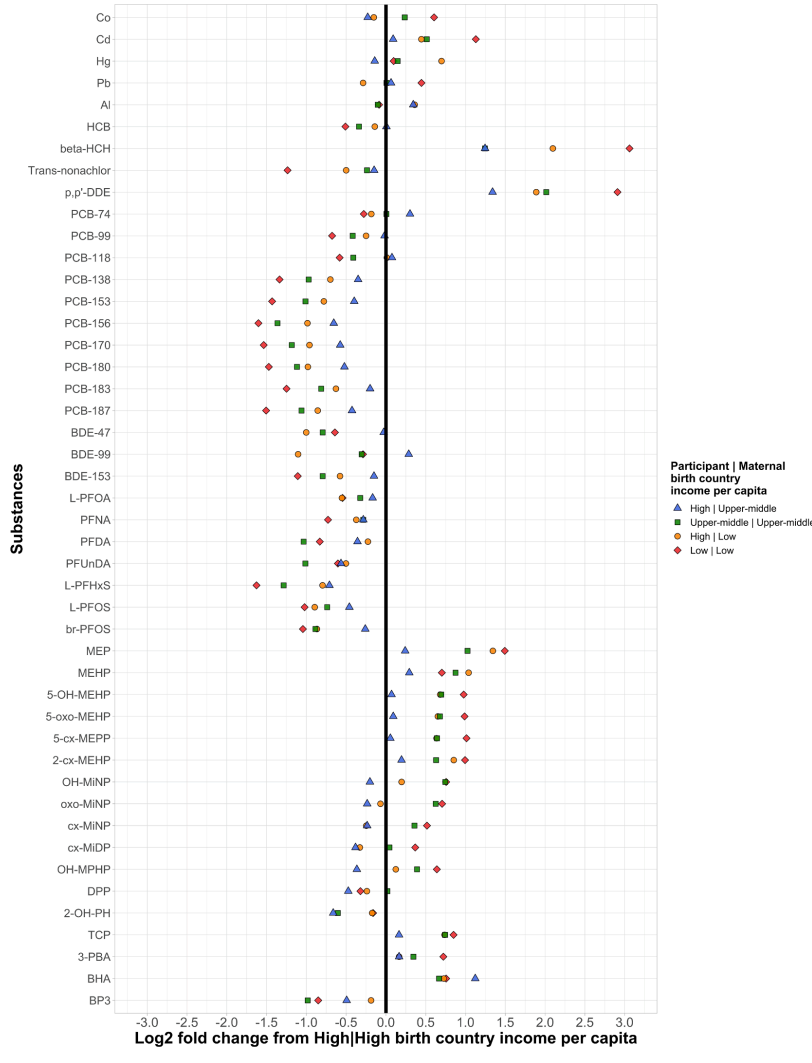

Fig. 3. Graph demonstrating the $\log_2$ converted fold-change of the estimated adjusted mean (EAM) substance concentrations for the different Participant/Maternal birth-country income per capita groups in comparison to the EAM concentration of the reference group: 'High | High income' (black line set at 0). Points to the left of the reference indicates negative fold-change whilst points to the right of the reference indicates positive fold-change. Adjusted for participant age, gender, maternal and paternal education level, and place of living (latitude and longitude). Only substances with significant association (p-values $\leq$ 0.05) with the Participant/Maternal birth-country income variable were included. Log_2 −0.5/0.5 corresponds to a fold-change of 1.4, −1/1 a 2-fold change, −2/2 a 4-fold change and −3/3 an 8-fold change.

correlations between element concentrations observed in RMA (Pineda et al., 2023). Chlorinated POPs showed a high degree of similarity of significant determinants, mainly; age, gender, participant/maternal birth country income per capita, parental education, and longitude (Fig. 2), in line with the moderate to very strong correlations between serum concentrations of the chlorinated POPs (Pineda et al., 2023). In contrast, the brominated POPs (PBDEs) and the fluorinated POPs (PFASs) showed a larger variation in significant determinants, which is most probably a reflection of differences in physicochemical properties, production, and use of these substances (Sharkey et al., 2020; Weber et al., 2011).

Our results suggests that at least some of the exposures of the adolescents to phthalates/phthalate alternatives, PFRs, pesticides, bisphenols and other rapidly metabolised/excreted substances on an individual basis occurred consistently over time, enough to be detected in single spot urine samples from all participants. This 'persistent' exposure made it possible to observe demography-dependent variation in urinary concentrations. As expected by the strong relationship between concentrations of urine metabolites with the same "mother substance" in RMA (Pineda et al., 2023), metabolites of the phthalates DEHP and DiNP, and phthalate alternative DiNCH, to a large degree shared significant determinants (Fig. 2). However, there were phthalate substance-dependent differences in determinants, suggesting differences in exposure sources among these plasticisers. As with the phthalates, metabolites of PFRs and PAHs, as well as the bisphenols, did not show consistent in-substance group determinant patterns (Fig. 2).

3.1. Participant/maternal birth country income per capita level

We found birth country disparities in adolescent body burdens for many of the substances (Fig. 3 and S.16). To the best of our knowledge, previous human adolescent biomonitoring studies have generally not considered the birth country of both the participant and the mother in their exposome analyses. Moreover, classification of birth countries according to national gross income per capita has rarely been used. Taken together the results strongly suggest that participant/maternal birth country income per capita level should be considered in future populations studies looking at the human chemical exposome and possible health effect outcomes (Figs. 3, S.2 and S.16).

Compared to the reference High | High group, EAM concentrations of Co, Pb, and Al among the different participant/maternal birth country groups were never more than 1.5-fold ($\log_2$ 0.6) higher or lower (Figs. 3 and S.16). The largest difference was observed for Cd with the 'Low | Low' group having a more than 2-fold higher EAM concentration than the 'High | High' group (Fig. 3). Cd has a very long half-life in the body (Nordberg et al., 2015), and therefore exposure of participants in their birth countries before moving to Sweden most probably contributed to the observed birth country relationships. A 'maternal effect' was, however, suggested since the EAM of Cd among high-income country participants became 1.4-fold higher ($\log_2$ 0.5) going from a high maternal birth country income to a low maternal birth country income (Fig. 3). Maternal transfer of Cd during foetal life and breast-feeding period is low (Vahter et al., 2002), suggesting that maternal birth country affected exposure after these periods. In German children/adolescents (GerES V), the geometric mean of Cd in urine was higher among 3–17 years old participants with two-sided immigration background (both participant and parents) than among those with no immigration background (Vogel et al., 2021). Direct comparisons between RMA and GerES results however remain uncertain as results in GerES were not adjusted for other demographic determinants. For Cd, Pb and Al the additional determinants included in the secondary regression models (Table S.1) did not markedly change the significance level of the birth country associations (Fig. S.9).

Due to the long half-lives of many of the halogenated POPs (Grandjean et al., 2008; Li et al., 2022; Ritter et al., 2009; Sjödin et al., 2020; Xu et al., 2020), exposure of participants in their birth countries is expected to be detectable for a long time after moving to Sweden. The PCB, PBDE and PFAS EAM concentrations in serum of 'High | High' participants were at most about 3-fold ($\log_2$ 1.6) higher than the 'Low | Low' group (Figs. 3 and S.16). In contrast, beta-HCH and p,p'-DDE (Fig. 4) showed a staggering 8-fold ($\log_2$ 3) higher EAM concentration among the 'Low | Low group compared to the 'High | High' group (Fig. 3). Similarly, GerES V participants with no immigration background had lower p,p'-DDE concentrations and higher PCB concentrations in plasma than those with a two-sided immigration background (Bandow et al., 2020). Higher DDT and HCH exposures have been reported in tropical and lower-income countries than in many industrialised high-income countries, whereas the reverse has been observed for PCBs (Berg et al., 2017; Haraguchi et al., 2009). Shorter half-lives of the lower-chlorinated PCBs and PBDEs (EFSA, 2005; Sjödin et al., 2020), resulting in a higher contribution of more recent exposure sources such as indoor environments (Johansson et al., 2003; Meyer et al., 2013; Wingfors et al., 2006) may at least partially explain the less obvious birth country relationships for these substances (Fig. 3).

Additionally, EAM PCB concentrations among high income-country participants decreased with decreasing maternal birth country income levels, suggesting a maternal effect, whilst the reverse was observed for beta-HCH and p,p'-DDE (Figs. 3 and S.16). Our results fit well with the observations that early life exposures to many lipid-soluble chlorinated POPs occurs via the placenta to the foetus and to infants via breast-feeding (Karmaus et al., 2001; Lignell et al., 2011), that persist as body burdens well into adult life (Gallo et al., 2011; Glynn et al., 2007; Lignell et al., 2011; Wesselink et al., 2019). The lower-chlorinated PCBs, PBDEs and PFASs with less pronounced adolescent participant/maternal birth country effects also showed less obvious maternal effects (Figs. 3 and S.16). The additional determinants in the secondary models (Table S.1) did not markedly affect the significance levels of the birth country relations of POPs (Fig. S.9).

The relationships between PFAS concentrations and birth country were similar as those reported by Nyström et al. (2023), generally with the highest EAM concentrations found in the 'High | High' group (Figs. 3 and S.16). Similarly, GerES V participants with no immigration background had higher PFOA concentrations in plasma than those with two-sided immigration background, whereas PFHxS and PFOS showed no associations (Duffek et al., 2020).

There were birth country-dependent disparities also in adolescent exposure to some metabolites of phthalates, PFRs, PAHs and the pesticides chlorpyrifos and pyrethroids, and the antioxidant BHA and the UV filter BP-3 (Figs. 3 and S.16). In the case of theses rapidly excreted/metabolised substances, it is likely that the participant/maternal birth country variable was a proxy for birth country-related household habits and living conditions in Sweden, including: diet, product usage, and housing, significantly affecting recurrent exposure among the adolescents. Metabolites of the phthalates DEP and DEHP all followed similar patterns in which the 'Low | Low' group had 2 to 3-fold ($\log_2$ 1–1.5) higher EAM concentrations compared to the 'High | High' group (Fig. 3). Results were more varied for the other phthalate metabolites but in general the 'Low | Low' group had the highest EAM concentrations. Similarly, GerES V participants with two-sided immigration background had higher DEP, DEHP, DiNP and DiDP metabolite concentrations in urine than those born in Germany with German born parents (Schwedler et al., 2020b). Although direct comparisons between RMA and GerES remain uncertain due to the non-adjusted immigration results in GerES, there appears to be similar exposure pathways involved in birth country inequalities of phthalate exposure of adolescents in Sweden and Germany. Moreover, in both RMA (Fig. 2) and GerES V, no birth country-related differences were observed in urine concentrations of BPA, and phthalate metabolites MBzP and MBP, and the metabolites of the phthalate alternative DiNCH (Schwedler et al., 2020a; Tschersich et al., 2021). The phosphorous flame-retardant metabolite DPP, PAH metabolite 2-OH-PH and the UV-filter BP3 generally showed a trend of highest EAM concentrations in the 'High | High' group, whereas the reverse was

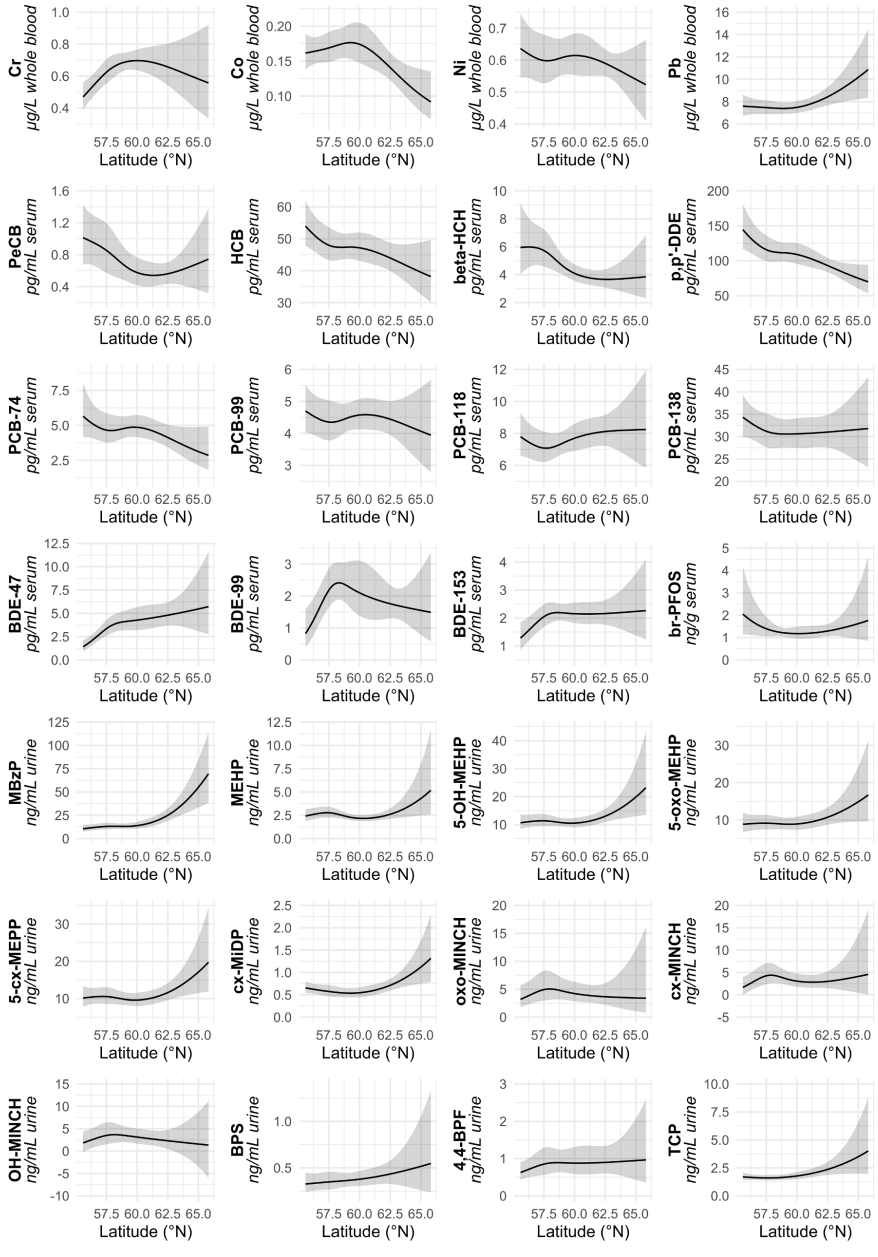

Fig. 4. Associations between concentrations of substances given at the normal scale and latitude of the home address. The solid line represents the estimated regression line whilst the shaded area represents the 95 % confidence interval. Determined in ordinal regression analyses with participant age, gender, maternal and paternal education level, place of living (longitude) and participant/maternal birth country per capita income as covariates in the regression models. Only substances with statistically significant associations with latitude ($p \leq 0.05$) are included in this figure.

evident for the pesticide metabolites TCP and 3-BPA and for the preservative BHA (Fig. 3). In GerES V no immigration status relationships were observed for 2-OH-PH and BP3. In RMA, inclusion of the additional determinants in the secondary models (Table S.1) did not markedly alter the significance levels of the birth country associations for phthalate and PAH metabolites, with the exception of cx-MiNP (Fig. S.9). The addition of ice cream consumption and season of sampling to the secondary model made the association non-significant, with ice cream consumption showing a significant association with cx-MiNP and greater share of the variance in the secondary model (Table S.127).

3.2. Gender, age and parental education

Many of the measured substances showed significant relations with gender, age and parental education (proxy of socioeconomic status) (Fig. 2) after adjustment of the associations with the other demographic variables. The differences in EAM concentrations between males and females were less than 1.5-fold ($\log_2$ 0.6) for many of the substances (Figs. S.4 and S.15). The largest gender inequality in EAM concentrations were observed for L-PFHxS (≈1.7-fold higher in males), br-PFOS (≈1.5-fold higher in males), the DEP metabolite MEP (≈1.7-fold higher in females), BHA (≈2-fold higher in females) and BP3 (≈2.3-fold higher in females) (Figs. S.4 and S.15). Food is a major source of exposure to some of the elements and POPs in Sweden (Livsmedelsverkets, 2017). Male adolescents having generally higher food consumption (Shomaker et al., 2010), may at least partially explain the higher concentrations of Hg, Pb, and halogenated POPs in RMA males compared to the female participants (Figs. S.4 and S.15). Due to these gender differences, a higher proportion of RMA males exceeded human biomonitoring guideline values (HBGVs) of Pb, HCB and PFOS compared to females (Pineda et al., 2023).

Direct comparisons of the RMA gender results with those from other studies are somewhat hampered by differences in adjustment for other demographic/life-style covariates. Nevertheless, Pb, chlorinated POPs and some PFASs were higher in males than females among the Flemish adolescents in FLEHS IV (Schoeters et al., 2022), as also reported for PFASs in a HBM4EU study with multiple EU countries included (also a subsample of RMA) (Richterová et al., 2023). This difference may in part be due to elimination of PFAS among females because of menstrual bleeding (Upson et al., 2022), although it has been speculated that other mechanistic differences in toxico-kinetics between males and females may be involved and initiated during puberty (Wu et al., 2015). Moreover, as in RMA, FLEHS IV females also had higher body burdens of Cd than males (Schoeters et al., 2022). PBDE concentrations in FLEHS were to a large degree non-detectable (Schoeters et al., 2022), whereas the use of estimated concentrations in the statistically analyses of RMA results suggested higher BDE-153 concentrations among males (Figs. 2 and S.15). In RMA, further adjustment for additional determinants in the secondary regression models (Table S.1) did not influence the p-values of the significant gender relations, except for Cd. The gender relation of Cd became non-significant, which appeared to be explained by gender differences in smoking (source of Cd exposure) and iron status (affects Cd toxicokinetics) (Bárány et al., 2005) (Fig. S.8; Table S.14).

Female participants had higher EAM concentrations of many substances measured in urine compared to males (Figs. 2, S.4 and S.15), suggesting day-to-day gender inequality in exposure. This could for instance entail higher use of cosmetics/hygiene products among female participants (Harley et al., 2016; Schoeters et al., 2022; Thakore, 2014; Wnuk et al., 2022), leading to the observed higher average female exposures to the phthalate DEP, the preservative BHA and the UV-filter BP3 (Fig. S.4). Similarly as in RMA, FLEHS IV females had higher concentrations of the phthalate metabolites MEP, MBP, and DEHP metabolites, and no gender association with MBzP (Fig. 2) (Schoeters et al., 2022). In contrast, FLEHS IV reported higher BPA concentrations among female participants whereas no gender relation of BPA was observed in RMA (Fig. 2). BPS concentrations were to a large degree not detectable in FLEHS IV, whilst in RMA, females had higher concentrations than males (Fig. S.4). Differences regarding associations between gender and PAH, PFR and pesticide metabolites were also observed between RMA (Fig. S.4) and FLEHS IV (Schoeters et al., 2022). Further adjustment for the additional determinants in the secondary regression models (Table S.1) did not markedly change the significance levels of the gender relations of the urine substances (Fig. S.8).

RMA participants had an age difference of on average 6 years and a maximum difference of 11 years. This may be considered as too small of an age difference for detection of significant relationships between substance concentrations and age. Nonetheless, a significant age-dependent increase in concentrations were observed for Se, Cd, p,p'-DDE, PCBs, BDE-47, BDE-153, the DEP metabolite MEP, BPS, 4,4-BPF, 2-OH-PH, TCS, BHA and BP3, whereas concentrations of Mn, Al, PFHxS, DEHP metabolites, the PFR metabolite DBP, and the pyrethroid metabolite 3-PBA decreased with increasing age (Fig. S.14). Among the elements, increases/decreases in EAM concentrations over the 11 years ranged from 1.1-fold to about 1.4-fold for Mn (decrease), Se (increase) and Al (decrease), whereas EAM concentrations of Cd increased ≈2-fold (Fig. S.14). The chlorinated and brominated POP EAM concentrations increased 1.2 to 1.9-fold, with BDE-153 showing the largest increase with age (Fig. S.14). Positive age associations of Cd and some chlorinated and brominated POPs could in part be due to the long half-lives of these substances in the human body, causing bioaccumulation with increasing age (Nordberg et al., 2022; Sjödin et al., 2020; Verner et al., 2009).

PFASs displayed an absence of significant trends with age (Fig. 2), despite having half-lives of several years (Li et al., 2022). The only exception was for PFHxS (Fig. S.14), which decreased with increasing age. In the context of RMA, the observed inverse relation with age is presumably influenced by the youngest participants (5th-graders) hailing from two schools situated in regions with elevated historical exposure to PFHxS through drinking water, specifically in Uppsala and Ronneby (Nyström et al., 2022). Consequently, 5th graders to a larger degree exceeded HBGVs of PFAS (Pineda et al., 2023).

When including the additional determinants in the secondary regression models (Table S.1), the positive age associations of PCBs became non-significant (Fig. S.7), suggesting that the additional significantly associated determinants; alcohol consumption (PCB-99, -118, -138, -183), BMI (PCB-99, -118, -138, -156, -170, -180, -183, -187), seafood consumption (PCB-99, -187), breastfeeding early in life (PCB-138, -156, -170, -180, -183, -187), and season of sampling (PCB-99, -118) to a large degree explained the age relations (Tables S.40-S.67). Additionally, the inverse age association of Al also became non-significant with the inclusion of ferritin levels and sampling season as additional variables (Fig S.7 & Table S.23).

The varying age relationships among substances measured in urine, even within chemical groups of similar substances, such as phthalates, (Fig. S.14) most probably reflect age-dependent differences in use of consumer products, dietary habits and living conditions affecting exposure (Bastiaensen et al., 2021b; Schwedler et al., 2020b; Tschersich et al., 2021). The largest increase in EAM concentrations during the 11-year age span was observed for BP-3 (≈4-fold), 4,4-BPF (≈4-fold) and MEP (≈3.5-fold) whilst the largest decrease was observed for the DEHP metabolites (≈1.4 – 1.8-fold) (Fig. S.14).

Unlike participant/maternal birth country income per capita level, we decided to keep the parental education determinants separated even though it could be argued that the maternal and paternal education levels are similar enough to potentially cause multicollinearity issues within the models. We justify the separation mainly due to there being only moderate correlation (Fig. S.3) and previous evidence indicating that there may be perceivable maternal and paternal relation differences at least within PFASs substances (Glynn et al., 2020), and therefore potentially other substance groups. The differences in EAM concentrations between parental 'higher education' and the other educational groups were not more than 1.5-fold (Figs. S.5, S.6, S.17 and S.18).

Participants with the highest maternal/paternal education levels generally had the highest EAM concentrations of chlorinated POPs (Figs. S.5 and S.6), similar to results observed in FLEHS IV when comparing to household education levels (Schoeters et al., 2022). In RMA, none of the elements and PBDEs showed relationships with parental education level, except Hg (paternal education) (Fig. S.5). Among PFASs and substances measured in urine, only PFUnDA, PFHxS, MEP, MBzP, BPS, 3-PBA and TCS were associated with maternal and/or paternal education levels (Fig. 2). In a HBM4EU study, adolescents with the highest household education level generally had the highest concentrations of PFOA, PFNA, PFHxS and PFOS (Richterová et al., 2023), which was not observed in the present study. As in the case of parental education levels, participant/maternal birth country income level is most probably a proxy for socio/economic status that could influence chemical exposure. In RMA, maternal education levels showed a weak positive correlation with participant/maternal birth country income levels, which may in part influence the mutually adjusted birth country and education level results. (Fig. S.3). In the HBM4EU the household education PFAS results were not adjusted for birth country income levels (Richterová et al., 2023).

Similar to RMA, Flemish adolescents with a high household education level had the lowest concentrations of MEP and the highest of PBA-3 (Schoeters et al., 2022). In RMA, HCB, L-PFHxS, MEP and MBzP were only related to maternal education and Hg, β-HCH, PCB-74, BPS, 3-PBA and TCS with paternal education (Figs. S.5 and S.6), suggesting diverging paternal and maternal influence on exposures to these substances. Similarly as for maternal education level in RMA, urinary concentrations of MEP and MBzP were lowest among the HBM4EU adolescents with high household education level (Govarts et al., 2023). However, in contrast to RMA, this was also reported for the other phthalate metabolites included (Govarts et al., 2023).

The additional determinants included in the secondary regression models (Table S.1), made the association between maternal education and PCB-118 non-significant, including the significant additional variables alcohol consumption, BMI, and season of sampling (Table S.46). Paternal education level associations with PCB-74, PCB-118 and PFUnDA turned non-significant (Fig. S.11). In these cases, the significant additional variables were alcohol consumption (PCB-74, -118), seafood consumption (PFUnDA), BMI (PCB-74, -118), being nursed early in life (PCB-74), and season of sampling (PCB-74, -118) (Tables S.40, S.46, S.87).

3.3. Latitude and longitude

The latitudinal trends were longitude-adjusted to 15.4 °East (Fig. 1), traversing Sweden through the regions Götaland, Svealand and Norrland from south to north with a trend of colder climate going north (Pettersson et al., 2020). Moreover, most arable land is found within Götaland and Svealand, declining in Norrland from around 60°N with forest land dominating the landscape going further north (Lindblom, 2008). The regions south of Norrland exhibit higher population density and industrial activity (Bustos et al., 2020; IF-Metall, 2014; OECD, 2020). This is reflected in the RMA study population, where a greater proportion resides below latitude 60°N (Fig. 1). Consequently, in some cases the latitudinal trend becomes increasingly uncertain as one moves further north in Sweden, as evidenced by inflated confidence intervals extending into Norrland (Fig. 4). Nevertheless, when looking at Fig. 4; Co, Ni, PeCB, HCB, β-HCH, p,p'-DDE, PCB-74 and PCB-99 showed a general trend of higher concentrations in southern Sweden compared to the north (Fig. 4). The reverse was indicated for Pb, BDE-47/-99/-153, MBzP, the DEHP metabolites, cx-MiDP, BPS and TCP (Fig. 4).

The longitudinal models were latitude-adjusted to 59°N (approximate to Stockholm) with the longitudinal *trans*-section of Sweden crossing from the Atlantic west coast to the Baltic Sea east coast of Sweden (Fig. 1). The longitudinal trends were most likely driven to an extent by the larger number of RMA participants living in the south of Sweden, with the urban Malmö and Göteborg regions in the southwest at the Atlantic coast, and the Stockholm region on the east coast of Sweden at the Baltic Sea (Fig. 1). Estimated longitudinal trends extending past ~18°E at the Baltic Sea coast are in some cases uncertain as noted by the inflated confidence intervals (Fig. S.19). A tendency of higher concentrations in the west than the east of Sweden was indicated for Cr, Pb and Al (Fig. S.19). The reverse was indicated for HCB, β-HCH, p,p'-DDE, some of the PCBs, L-PFHxS (to ~18°E), L- and br-PFOS, some DEHP metabolites, cx-MiDP, OH– MPHP, DPP and BBOEP (to ~18°E) (Fig. S.19).

Among the elements the largest contrast in EAM concentrations along the latitudinal gradient was observed for Co (≈2-fold) (Fig. 4). Plant-based foods is a major source of element exposure of the Swedish population (Livsmedelsverket, 2017) and the varying latitudinal trends for the elements (Fig. 4) may be due to regional differences in food consumption patterns of both domestically produced and imported foods, and/or regional differences in element concentrations in domestically produced food. Agricultural use of the fungicide HCB and insecticides HCH and DDT could contribute to the decreasing south-north trend of HCB, β-HCH, p,p'-DDE (Fig. 4), and for HCB also industrial/combustion pollution in the south (Chen et al., 2019). Among the chlorinated POPs, p,p'-DDE showed the most obvious difference in EAM concentrations decreasing ≈2.5-fold going south-to-north (Fig. 4). Similar gradients were observed in adipose tissue of food-producing bovines and pigs farmed within Sweden for HCB, p,p'-DDE and PCBs (Glynn et al., 2009). BDE-47 showed the largest difference between the lowest and highest latitudinal EAM concentration at ≈3.5-fold from south to north (Fig. 4). This corroborates the findings of a previous small study focusing on nursing women in Sweden which showed higher PBDEs concentrations in breast milk among women living in a northern Norrland municipality than in urban areas in southern/central Sweden (Glynn et al., 2011). The south to north trends of increasing concentrations observed for MBzP, DEHP metabolites and cx-MiDP (Fig. 4), may be due to a climate-dependent influence of exposure from the indoor environment (Bastiaensen et al., 2021a; Schwedler et al., 2020b). Moreover, a contributing factor could be the higher degree of urbanisation of Götaland and Svealand compared to Norrland affecting the type of housing in the regions. The largest difference in EAM concentration was observed for MBzP, being ≈5.5-fold lower in southern Sweden than in the north (Fig. 4).

As in the case of the south-north trends, elements showed diverging west-east gradients, further emphasising dissimilar exposure sources (Fig. S.19). Cr showed the largest west-east variation in EAM concentration, being ≈3-fold higher in the west (Fig. S.19). Almost all chlorinated POPs showed a tendency of lower concentrations in the western than in the eastern part of Sweden, in some cases with lowest concentrations in-between (Fig. S.19). The largest difference in EAM concentrations from west to east was observed for PCB-74 (≈8-fold), with a large uncertainty in the estimate for the eastern part of Sweden. The eastern Baltic Sea coast of Sweden is more highly contaminated with chlorinated POPs than the Atlantic west coast (Nyberg et al., 2015). Among the PFASs, west-east gradients varied somewhat, with the largest difference in EAM concentrations between west and east observed for br-PFOS (≈4-fold) with large estimate uncertainties in the east (Fig. S.19). In urine, MBzP, metabolites of DEHP and DiNP, cx-MiDP and OH-MPHP showed significant but varying mother substance group longitudinal trends (Fig. S.19). The differences in EAM concentrations going west to east ranged from ≈1.6-fold (some DEHP and DiNP metabolites) to ≈3.3-fold (OH-MPHP). As in the case of the latitudinal trends, the indoor environment may have influenced exposure to some phthalates. Unlike the latitudinal trends, climate-driven factors are less likely to be important for the longitudinal trends.

The additional determinants included in the secondary regression models (Table S.1) resulted in a non-significant latitudinal trend of Pb, PCB-99, PCB-118, and PCB-138 (Fig. S.12), including the significant determinants smoking (Pb), season of sampling (PCB-99, -118), alcohol

(PCB-99, -118, -138), seafood consumption (Pb, PCB-99), and BMI (PCB-99, -118, -138) within the models (Table S.20, S.43, S.46 and S.49), as well as breastfeeding early in life in the case of PCB-138 (Table S.49). Moreover, the longitudinal trend of PFNA and cx-MiNP became non-significant (Fig. S.13), with seafood consumption significantly associated with PFNA concentrations and ice cream consumption to cx-MiNP (Table S.81 and S.127).

4. Strengths and limitations

A major strength of the study is the RMA study population itself, with a population-based design encompassing the whole of Sweden (Fig. 1). The recruited population could be regarded as representative for the adolescent population in Sweden when looking at school type and size, education level and income of the parents, and with all types of municipalities represented (Moraeus et al., 2018). Moreover, the distribution of household incomes, parental education levels and country of birth of the adolescents (Sweden/outside Sweden) were similar when comparing RMA participants and the total population of school-attending adolescents in Sweden (Moraeus et al., 2018). However, limitations of representativeness include slightly more females than males among the participants, and lack of adolescents not attending school; mostly in the same age group as the 11th graders (Moraeus et al., 2018).

In addition to the targeted chemical analysis of a multitude of toxic substances from various chemical groups, the RMA survey also collected data on lifestyle and dietary habits. While self-reporting contributed to uncertainty in the statistical analyses, the collected data allowed for a comprehensive, representative and holistic analysis of the associations between toxic substance mixture burden within Swedish adolescents and socio-demographic factors. The use of ORMs enabled us to deal with the natural skewness of the substance concentration data and still incorporate outliers with extreme substance concentrations, without requiring transformation of the data.

Although the schools were selected to include a sample of adolescents representative of the Swedish population, due to the demographic distribution of Sweden, a larger proportion of the schools were located in the southern part of the country (Fig. 1). Moreover, RMA did not include data on the duration participants had lived in the study area and ultimately this factor could not be controlled for within the models. Regardless, we could predict latitudinal and longitudinal concentration patterns with a reasonable degree of confidence, suggesting this approach to be promising for studies of regional differences in human exposure to toxic substances outside of Sweden. Furthermore, we acknowledge there are some potential issues in using birth country income per capita as a determinant for exposure. Countries belonging to any given national income per capita bracket can be geographically distant from each other and also be in different stages of industrialisation despite having the same national income per capita classification. Additionally, we did not have data available on the time the participants and their mothers spent in their reported birth countries and are therefore unable to control for time not living in Sweden within the models.

There are some inherent issues with the analysis of substances in single spot urine samples. Namely, the substantially short half-lives of substances found in urine cause large day to day variation in concentrations (Philippat and Calafat, 2021). Nevertheless, in many cases we observed clear associations between substances measured in single spot urine samples and demographic determinants, suggesting that some exposure sources are likely to be consistent from day-to-day.

5. Conclusions

Our study offers unique insight into the chemical exposome, including various types of toxic substances, in a population undergoing a sensitive window of development (puberty). Our results show that there are gender, age, socio-economical (parental education), birth country and regional inequalities in toxic substance exposure among adolescents living in Sweden that should be considered in future risk management decisions aiming to reduce exposure. Further, the results show that UN sustainable development goal 10, "Reduce inequality within and among countries", was highly relevant for the RMA adolescents in Sweden, since we identified participant/maternal birth country income as the most impactful determinant. The use of birth country income per capita level as a base for grouping of birth countries showed to be a promising path for future studies of this important factor in exposure assessment and relations to health outcomes. The UN goal 5, Gender equality, was also highlighted by our results since gender disparities in concentrations of substances varied between substances/substance groups, most likely due to gender differences in exposure, but in some cases likely also due to sex differences in toxicokinetics. The within-country aspect of Goal 10 is also an important goal to consider in Sweden since we observed latitudinal and longitudinal patterns for some substances/substance groups, which can aid in identification of regional populations at risk of elevated exposure. Additional studies are needed to better characterise the underlying factors which drive associations between contaminant exposure and socio-demographic status.

CRediT authorship contribution statement

Sebastian Pineda: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Sanna Lignell:** Writing – review & editing, Writing – original draft, Supervision, Resources, Data curation, Conceptualization. **Irina Gyllenhammar:** Writing – review & editing, Writing – original draft, Supervision, Resources, Data curation, Conceptualization. **Erik Lampa:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology. **Jonathan P. Benskin:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Data curation. **Thomas Lundh:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Data curation. **Christian Lindh:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Data curation. **Hannu Kiviranta:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Data curation. **Anders Glynn:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.108618.

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QQ plots of all contaminant models S.1

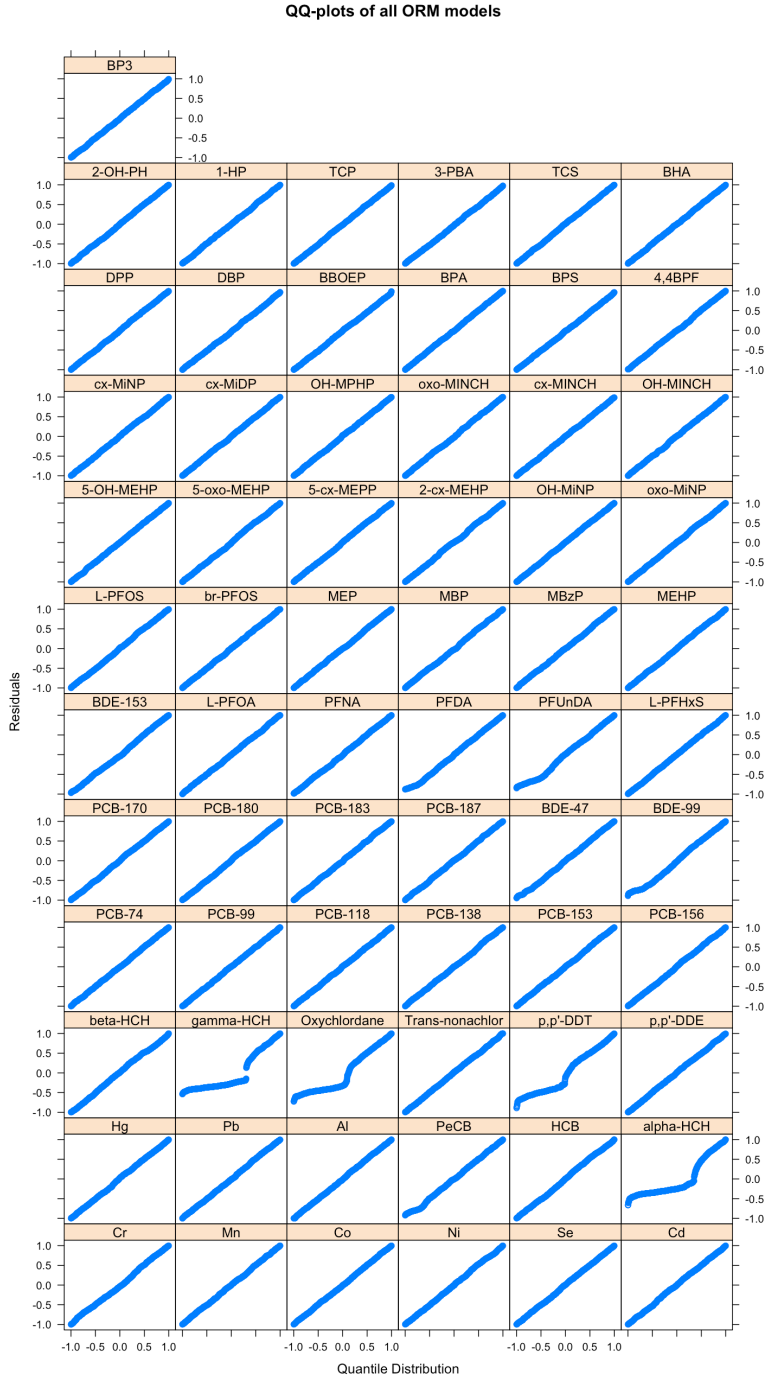

Figure S.1. Meta-plot showing quantile residuals on a uniform redistribution of all main ordinal regression models. QQ plots that deviate from a linear line suggest abnormal distribution and that the model will have poor 'goodness of fit' and more analysis uncertainty.

Chi^2 proportion figure S.2

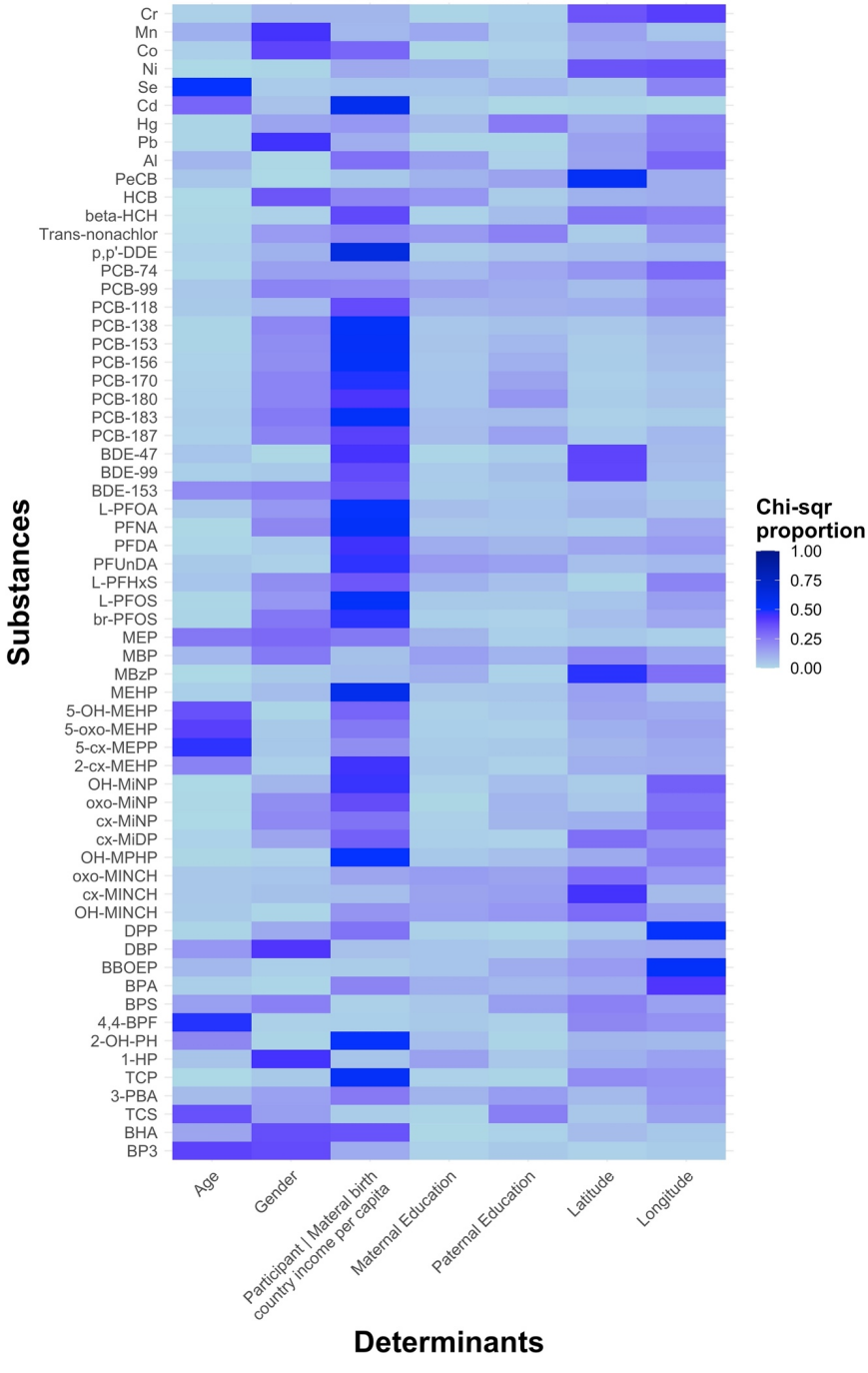

Figure S.2. Heatmap of the chi^2 proportion of the demographic determinants in each contaminant base model. Deeper shades of blue are equivalent to a higher proportional contribution of any given determinant (y-axis) to the total chi^2 of each substance model. High chi^2 proportion indicates a stronger main variable effect on the model compared to the other included variables, within that specific substance model.

Correlation matrix S.3

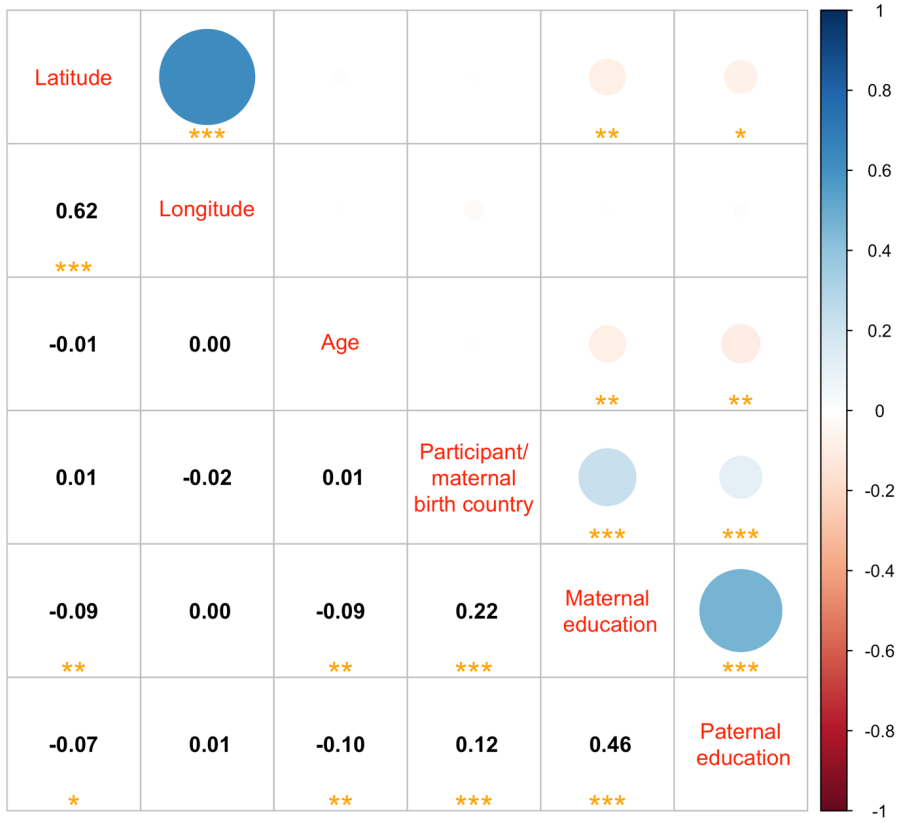

Figure S.3: Spearman's rank correlation coefficient matrix of all covariates used in the base models except gender. Correlation coefficients are given in text on the bottom-left and represented as graphical circles on the top-right. Significance levels (p-value) indicated as '' = 0.05, '**' = 0.01 and '***' = <0.0001.*

Fold-difference plots

Gender S.4

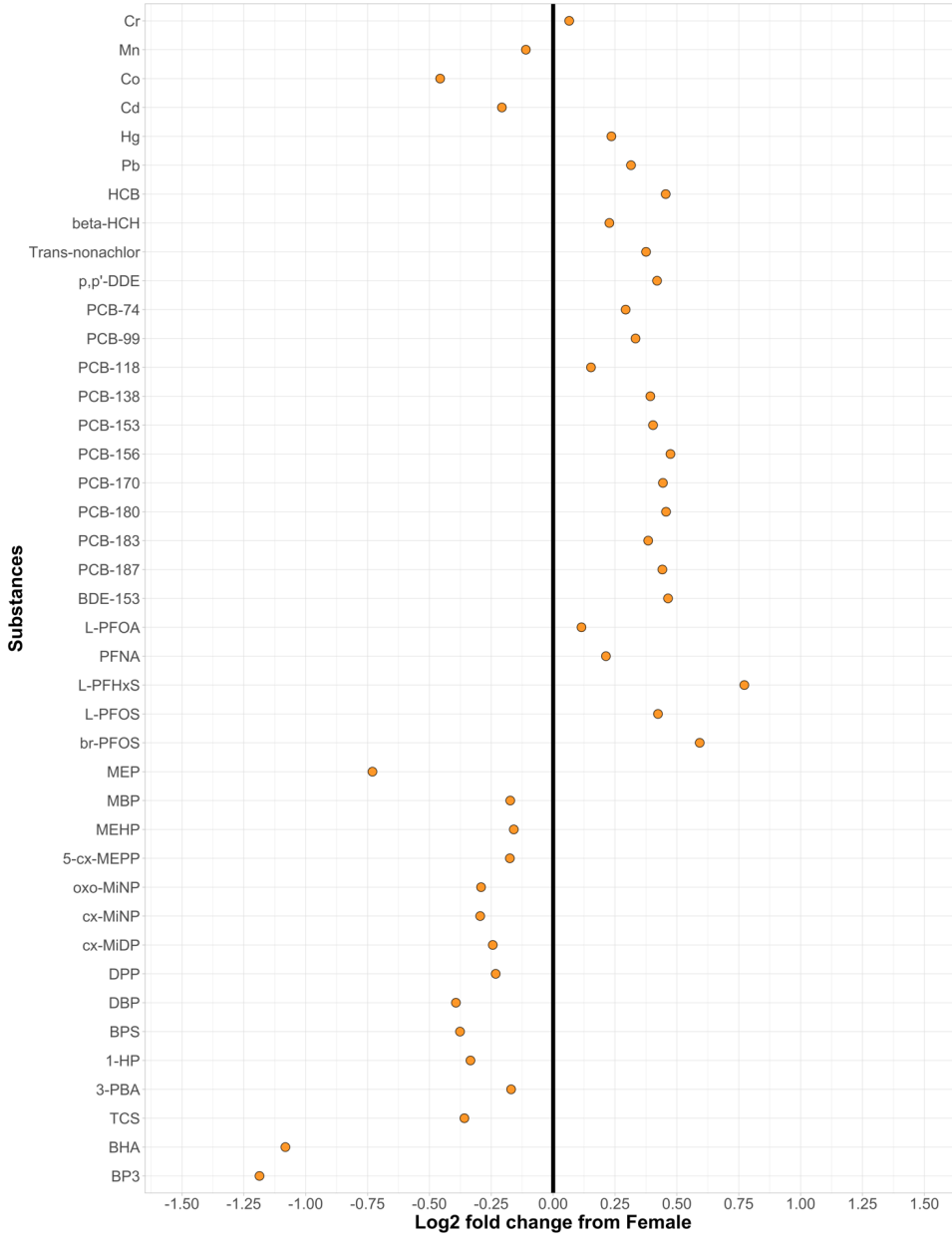

Figure S.4. Graph demonstrating the log_2 converted fold-change of the estimated adjusted mean (EAM) substance concentrations for the 'Male' gender group in comparison to the EAM concentration of the reference 'Female' group (black line set at 0). Points to the left of the reference indicates negative fold change whilst points to the right of the reference indicates positive fold change. Adjusted for age, P/M birth-country per capita income, maternal and paternal education level, and place of living (latitude and longitude). Only substances with significant associations (p-values ≤ 0.05) with the gender variable were included within this figure. Log_2 -0.5/0.5 represents a 1.4-fold change in comparison to the reference, -1/1 a 2-fold change and -1.5/1.5 a 2.8-fold change.

Maternal Education S.5

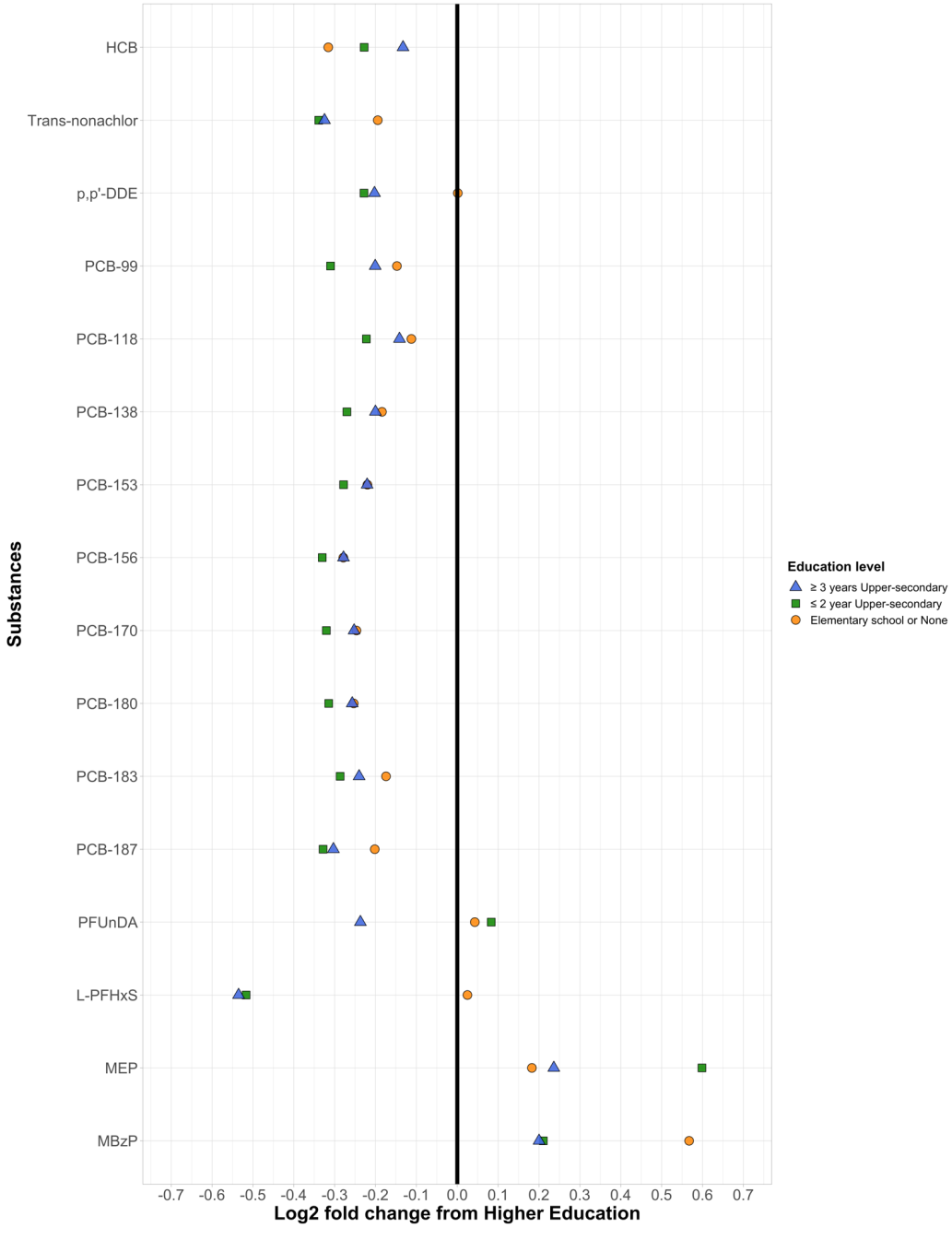

Figure S.5. Graph demonstrating the log_2 converted fold-change of the estimated adjusted mean (EAM) substance concentrations for the different maternal education groups in comparison to the EAM concentration of the reference 'Higher education' group (black line set at 0). Points to the left of the reference indicates negative fold-change whilst points to the right of the reference indicates positive fold-change. Adjusted for participant age, gender, P/M birth-country per capita income, paternal education level, and place of living (latitude and longitude). Only substances with significant associations (p-values ≤0.05) with the maternal education variable were included within this graph. Log_2 -0.2/0.2 represents a 1.1-fold change in comparison to the reference, -0.4/0.4 a 1.3-fold change and -0.6/0.6 a 1.5-fold change.

Paternal Education S.6

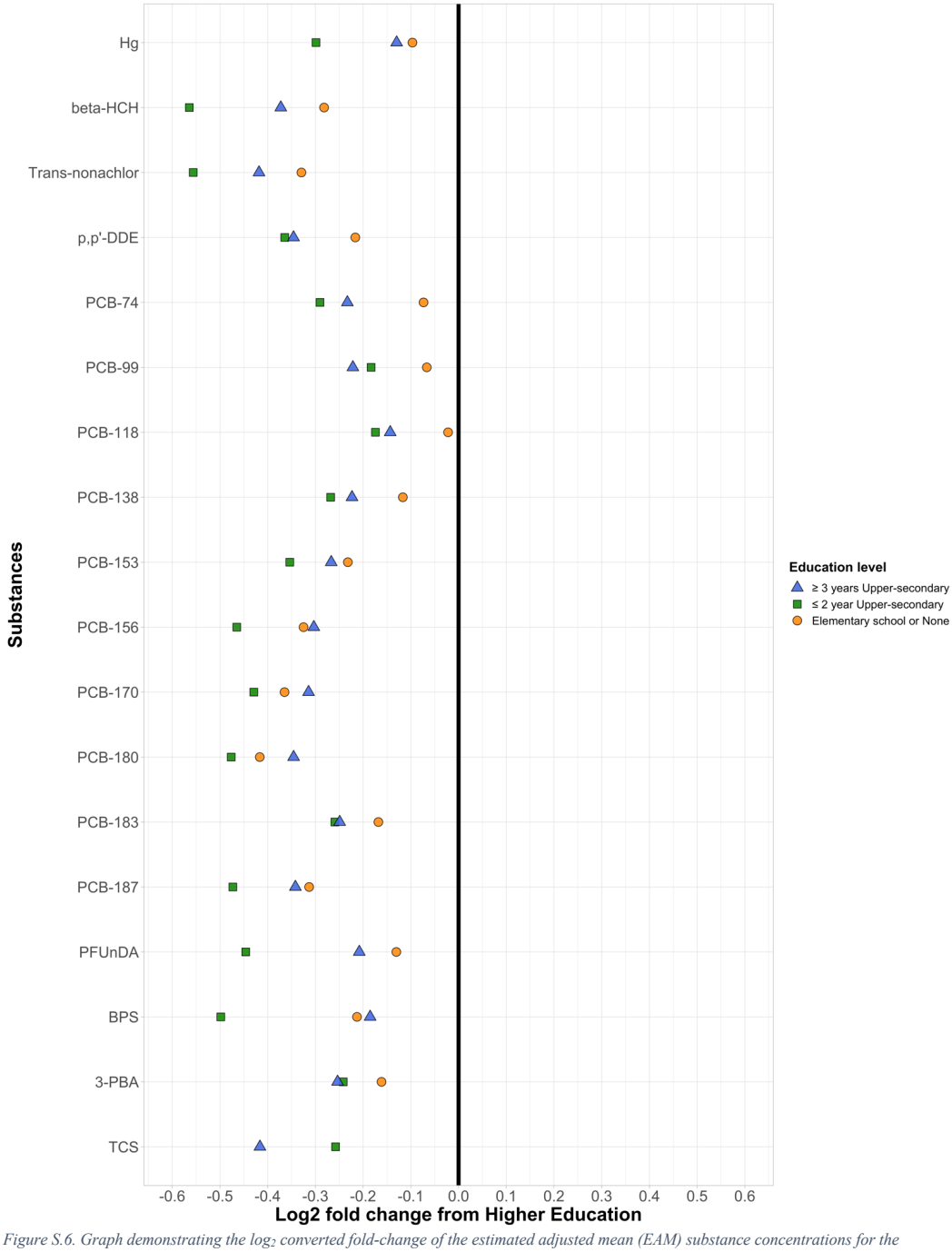

Figure S.6. Graph demonstrating the log_2 converted fold-change of the estimated adjusted mean (EAM) substance concentrations for the different paternal education groups in comparison to the EAM concentration of the reference 'Higher education' group (black line set at 0). Points to the left of the reference indicates negative fold-change whilst points to the right of the reference indicates positive fold-change. Adjusted for participant age, gender, P/M birth-country per capita income, maternal education level, and place of living (latitude and longitude). Only substances with significant associations (p-values ≤ 0.05) with the maternal education variable were included within this graph. Log_2 -0.2/0.2 represents a 1.1-fold change in comparison to the reference, -0.4/0.4 a 1.3-fold change and -0.6/0.6 a 1.5-fold change.

Comparisons plots

Age S.7

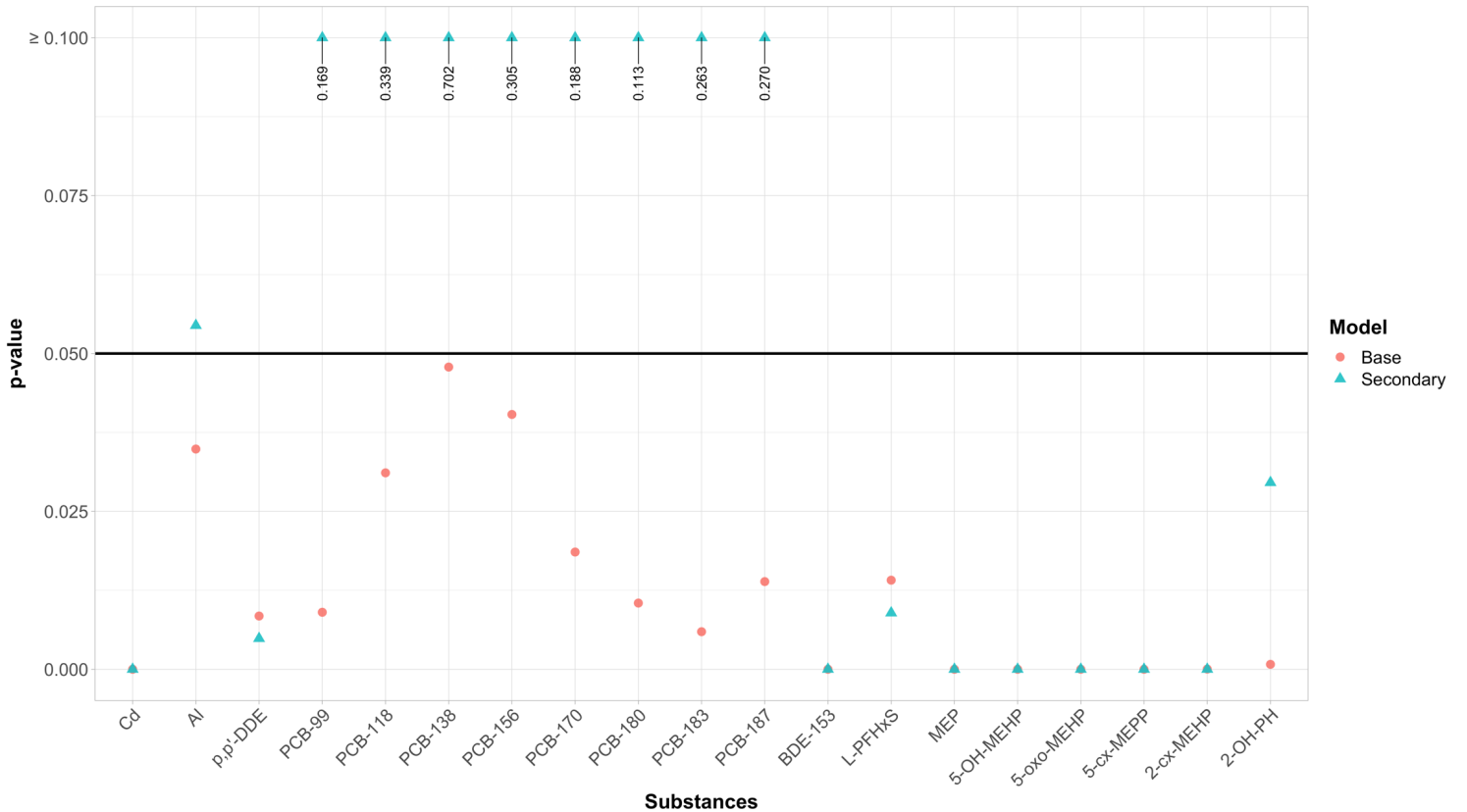

Figure S.7: Comparison plot of p-values for the associations between substance concentrations and the age variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1). Only substances with significant associations (p-values ≤0.05) with the age variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Gender S.8

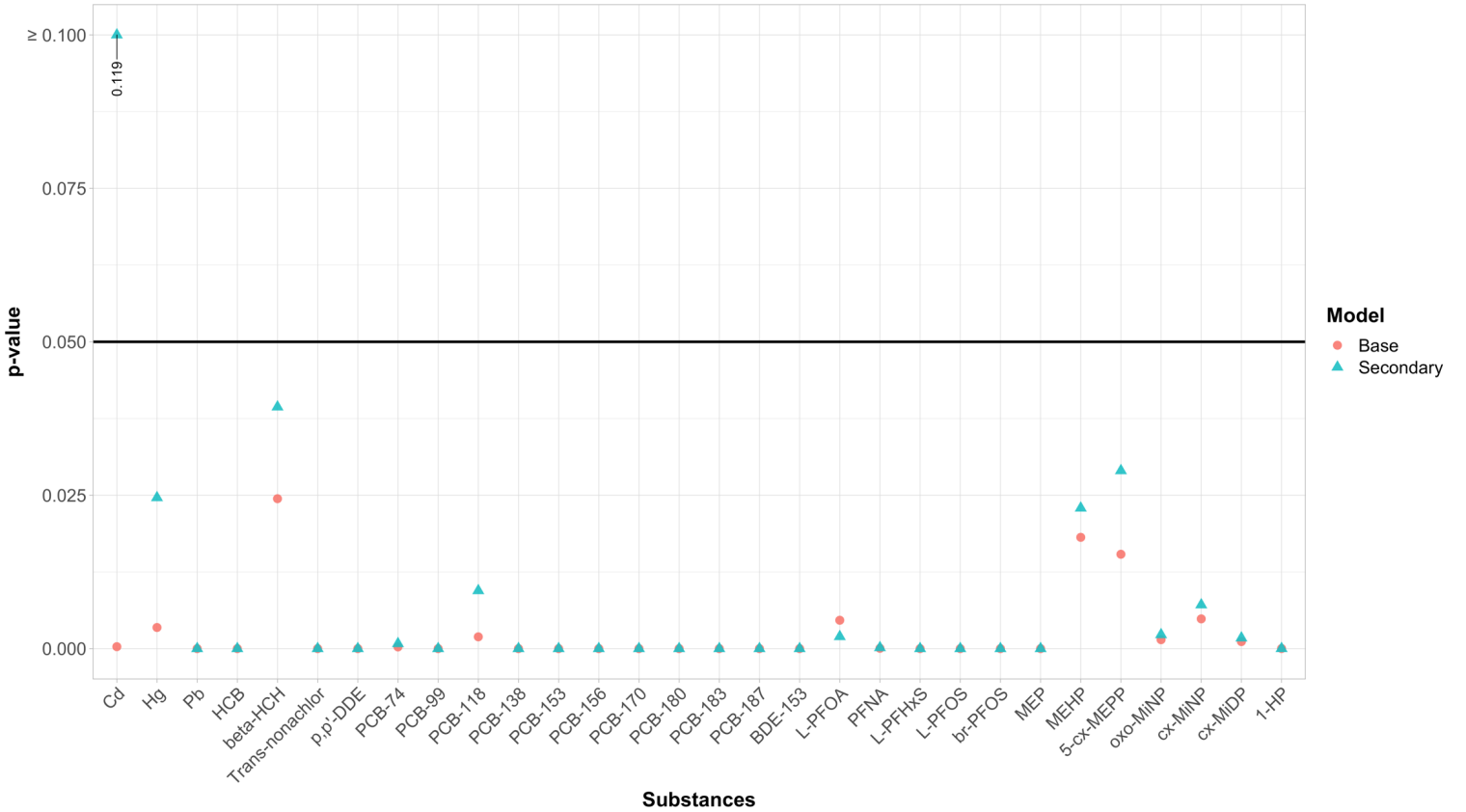

Figure S.8: Comparison plot of p-values for the associations between substance concentrations and the gender variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1). Only substances with significant associations (p-values ≤ 0.05) with the gender variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Participant/Maternal birth country income per capita level S.9

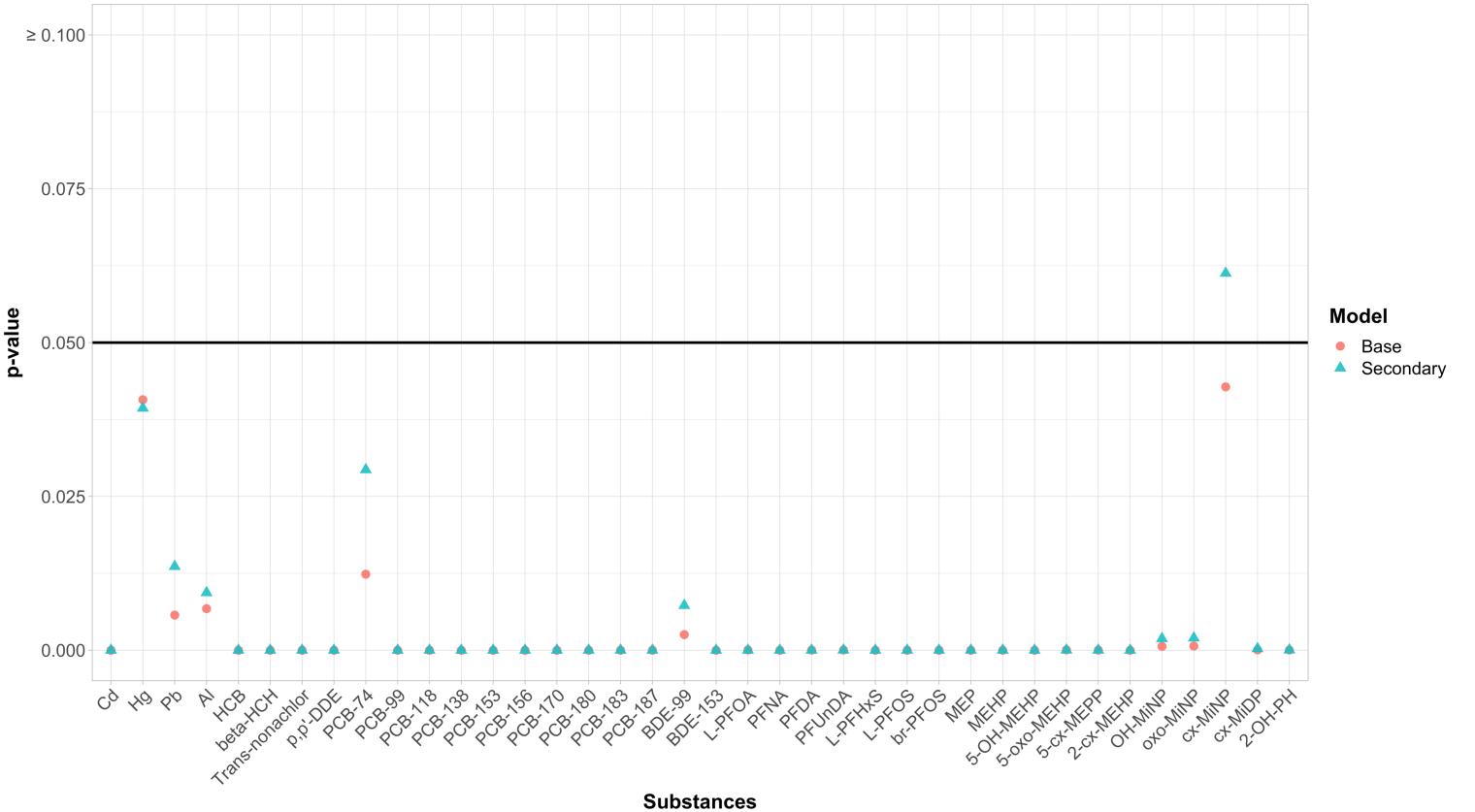

Figure 9: Comparison plot of p-values for the associations between substance concentrations and the participant/maternal birth country income per capita variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1). Only substances with significant associations (p-values ≤ 0.05) with the participant/maternal birth country variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Maternal Education S.10

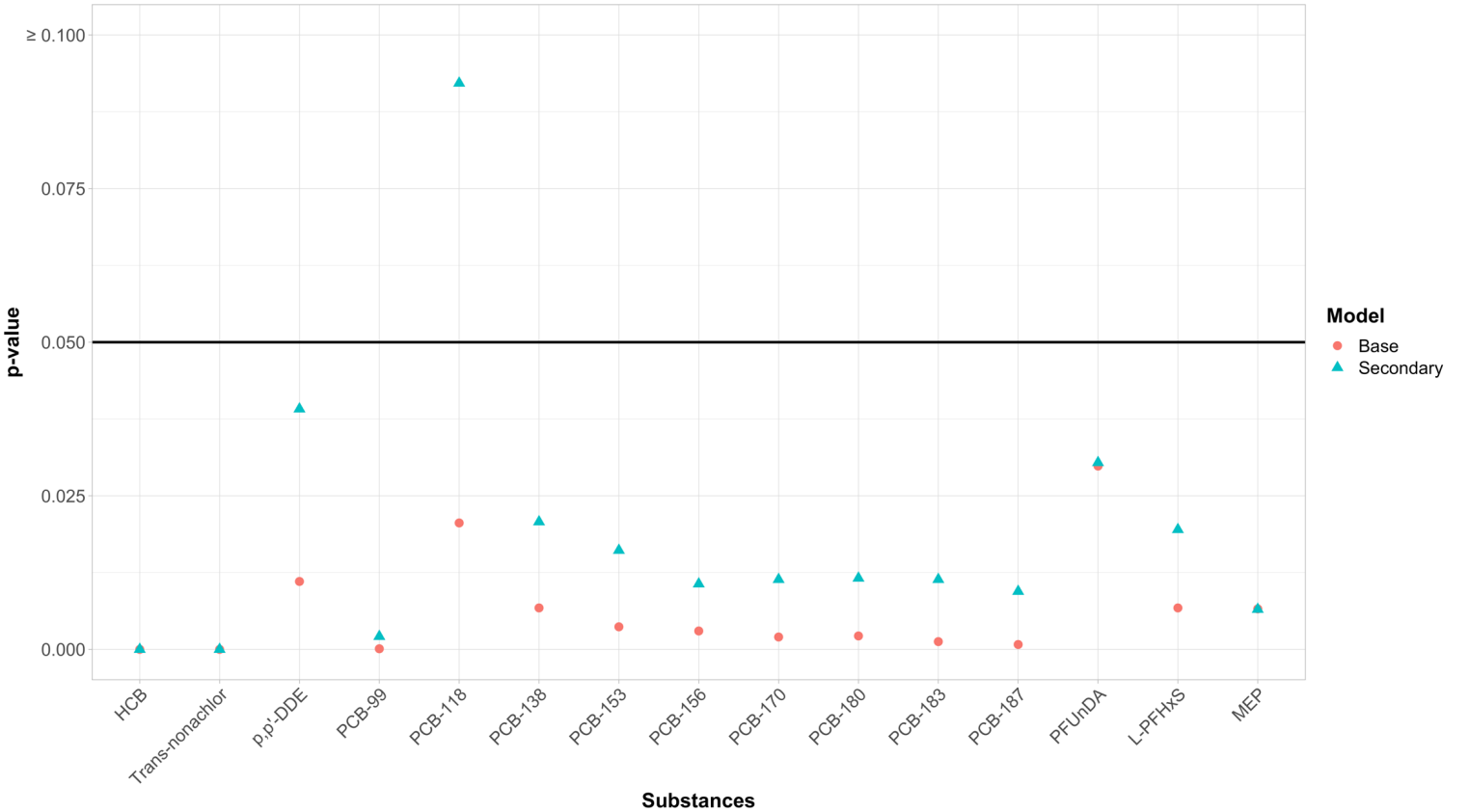

Figure S.10: Comparison plot of p-values for the associations between substance concentrations and the maternal education variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1. Only substances with significant associations (p-values ≤0.05) with the maternal education variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Paternal Education S.11

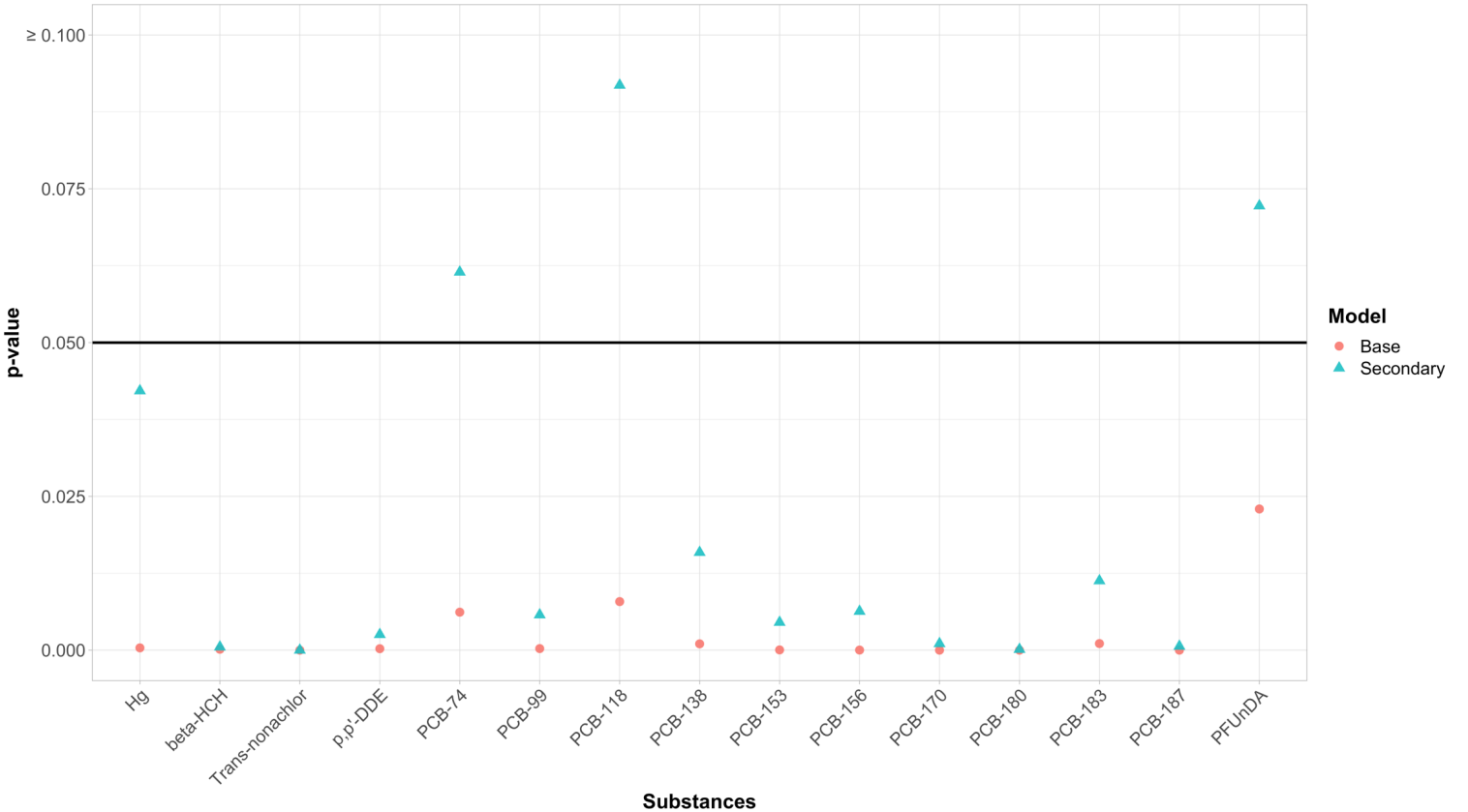

Figure S.11: Comparison plot of p-values for the associations between substance concentrations and the paternal education variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1). Only substances with significant associations (p-values ≤ 0.05) with the paternal education variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Latitude S.12

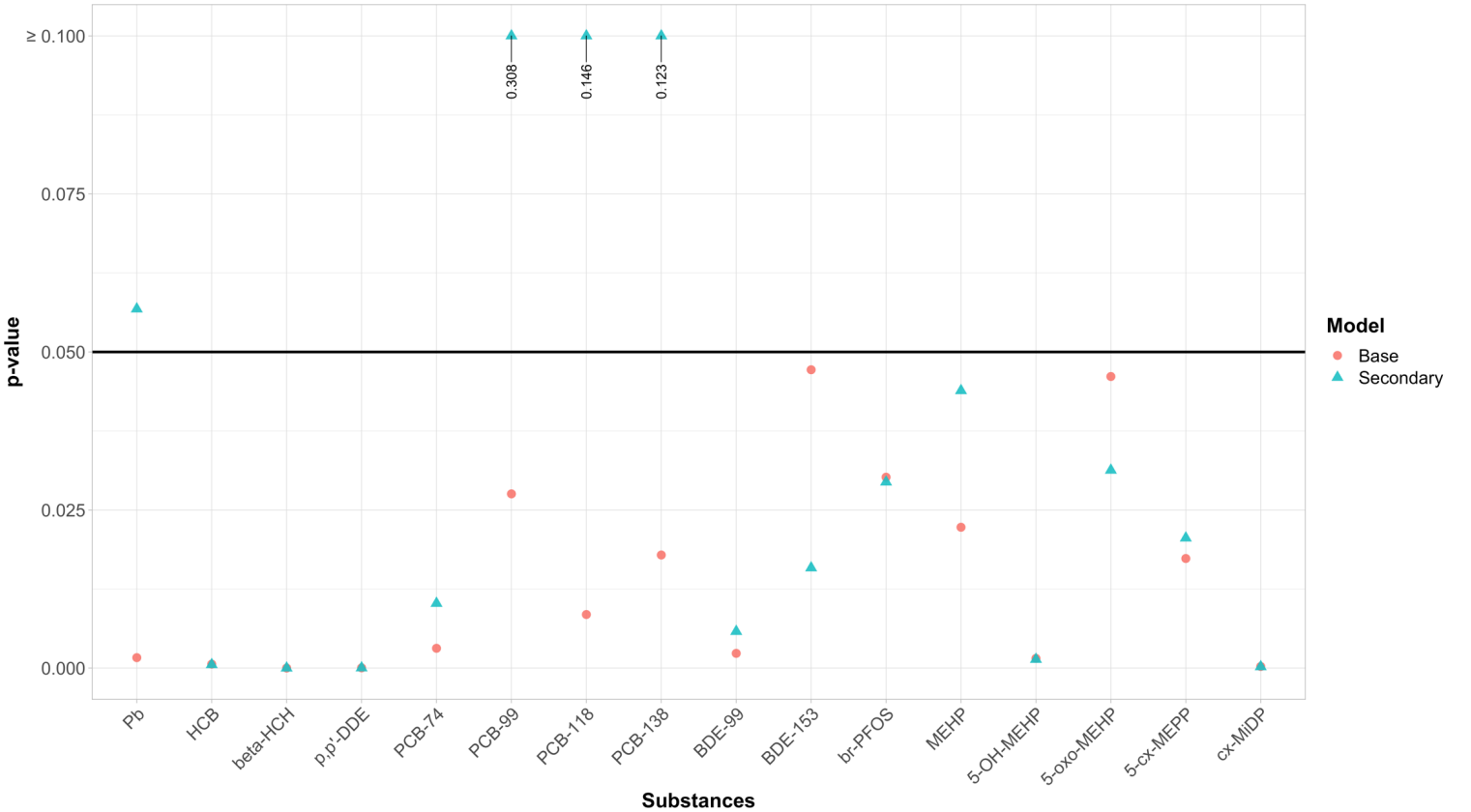

Figure S.12: Comparison plot of p-values for the associations between substance concentrations and the latitude of home address variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1). Only substances with significant associations (p-values ≤ 0.05) with the latitude variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Longitude S.13

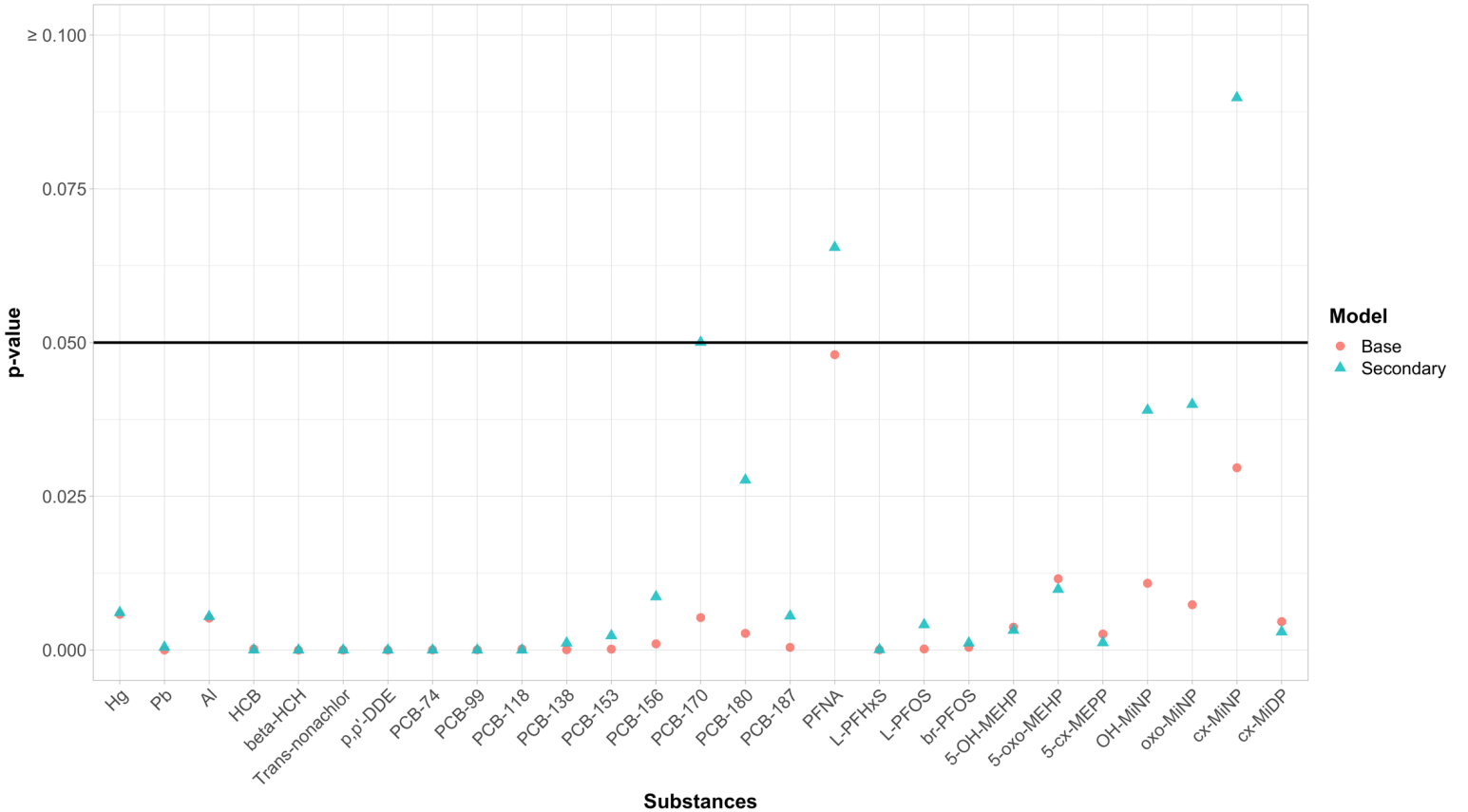

Figure S.13: Comparison plot of p-values for the associations between substance concentrations and the longitude of home address variable. Comparisons are between the p-values for associations obtained in the base model including only the demographic determinants and those from the secondary regression models also including additional covariates (Table S.1). Only substances with significant associations (p-values ≤ 0.05) with the longitude variable in the base model and with available additional covariates in the secondary models (Table S.1) were included within this graph.

Model analysis

Age S.14

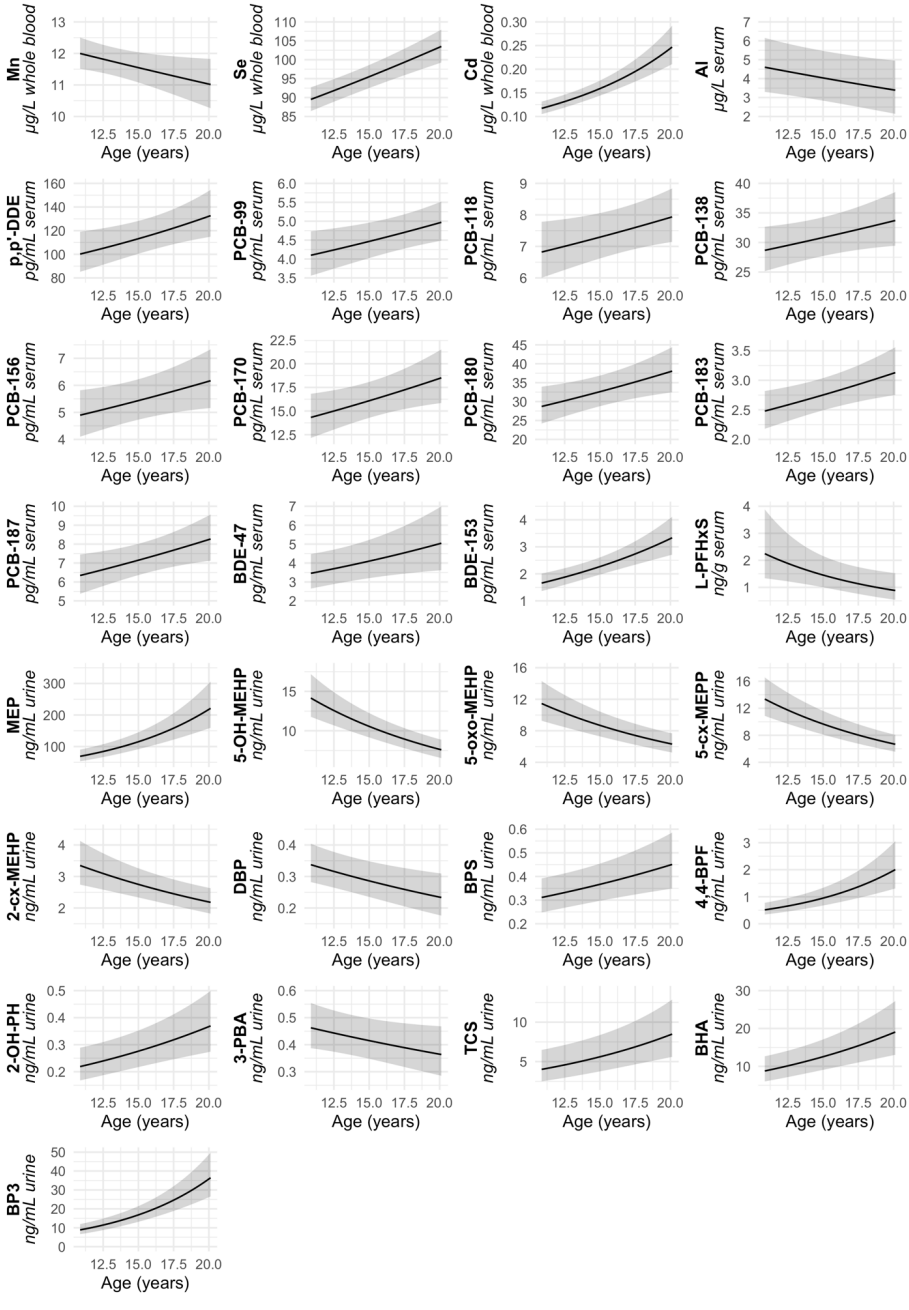

Figure S.14: Associations between concentrations of substances given at the normal scale and age of the participants. The solid line represents the estimated regression line whilst the shaded area represents the 95% confidence interval. Determined in ordinal regression analyses with participant gender, maternal and paternal education level, place of living (latitude and longitude) and participant/maternal birth country as covariates in the regression models. Only substances with statistically significant associations with age ($p \leq 0.05$) are included in the figure.

Gender S.15

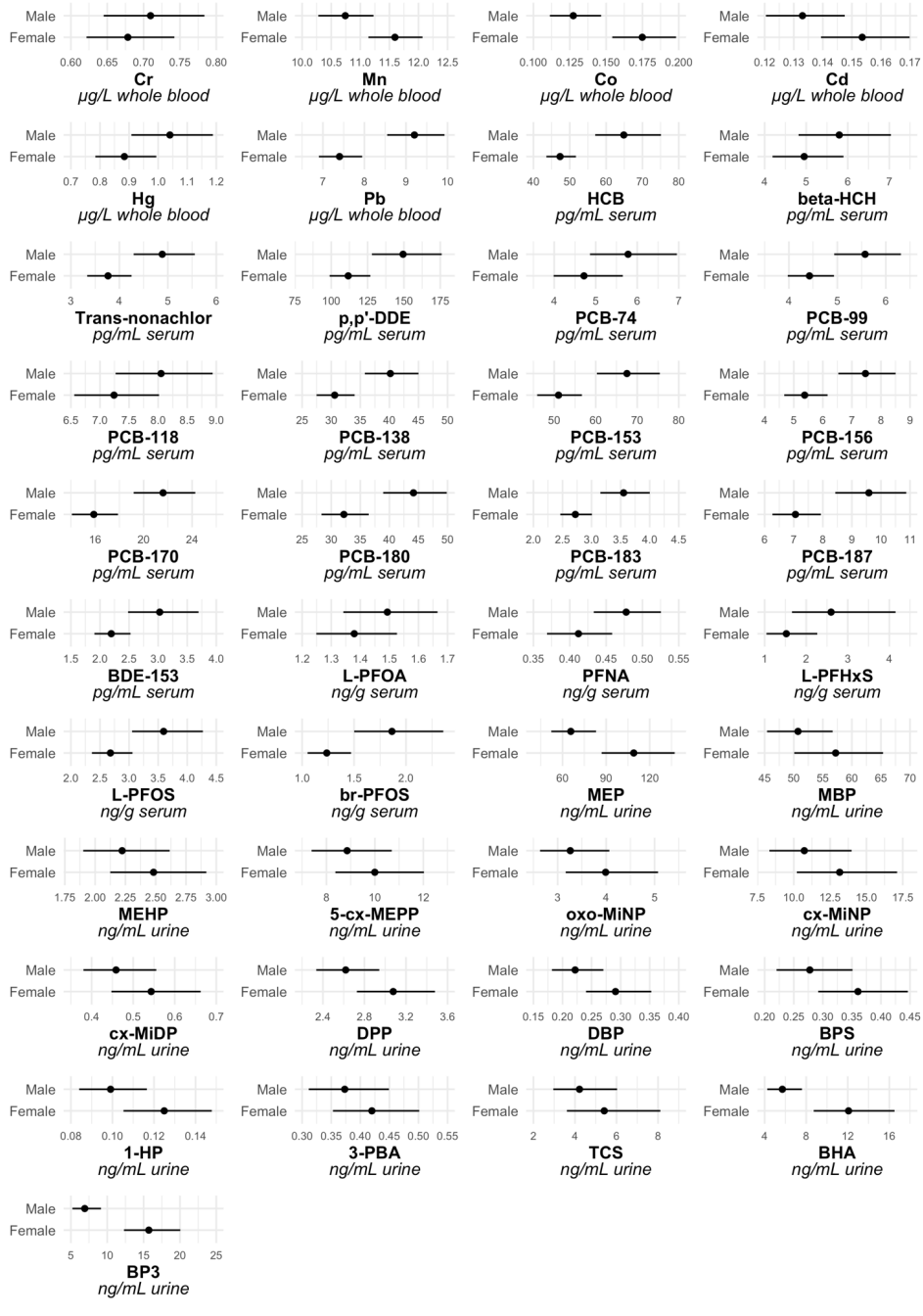

Figure S.15: Associations between concentrations of substances given at the normal scale and gender of the participants. The solid circle represents the estimated adjusted means concentration whilst the solid line represents the 95% confidence interval. Determined in ordinal regression analyses with participant age, maternal and paternal education level, place of living (latitude and longitude) and participant/maternal birth country per capita income as covariates in the regression models. Only substances with statistically significant associations with age ($p \leq 0.05$) are included in the figure.

Participant/maternal birth country income per capita level S.16

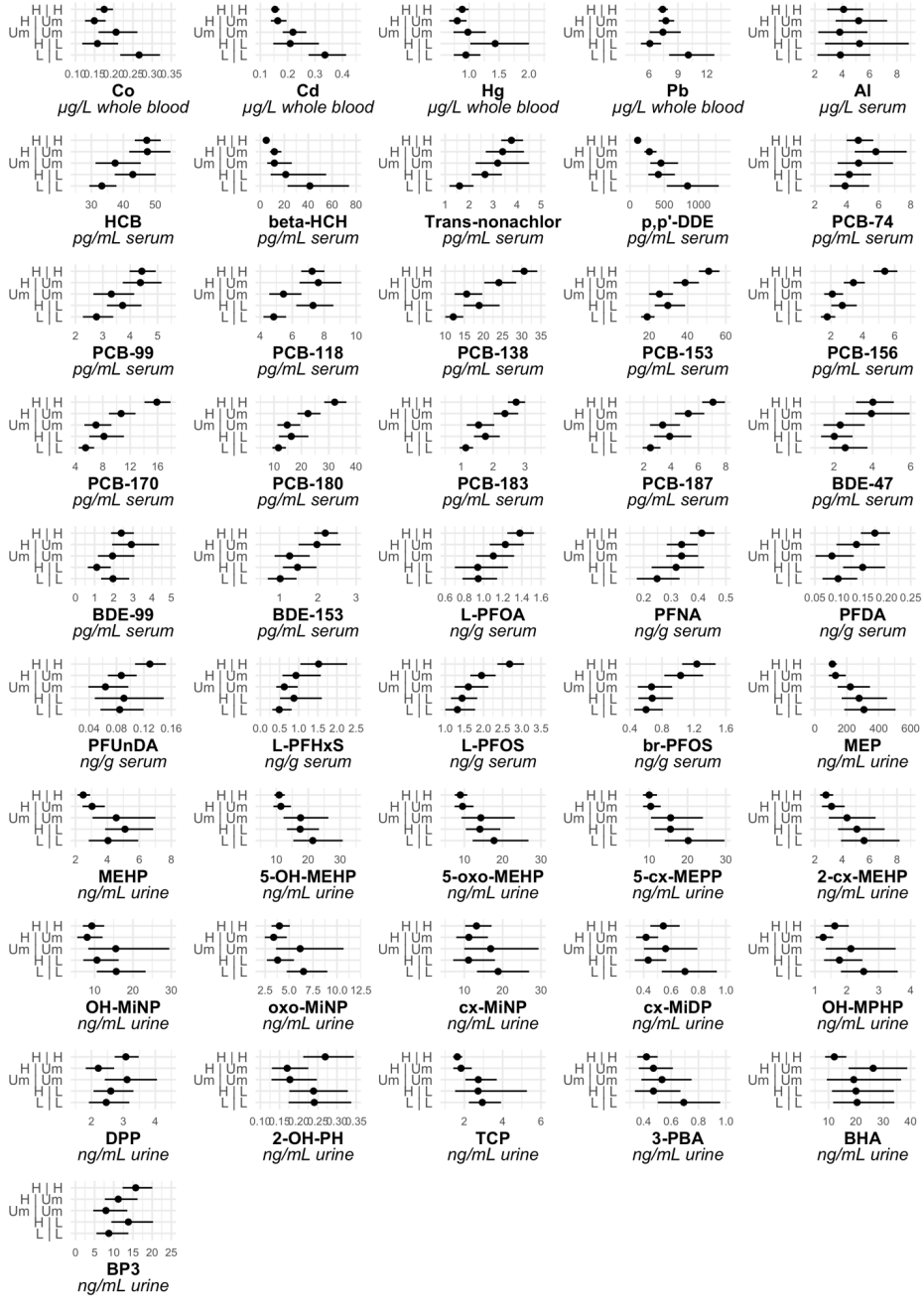

Figure S.16: Associations between concentrations of substances given at the normal scale and participant/maternal birth country income per capita levels. The solid circle represents the estimated adjusted means concentration whilst the solid line represents the 95% confidence interval. Determined in ordinal regression analyses with participant age, gender, maternal and paternal education level, and place of living (latitude and longitude) as covariates in the regression models. Only substances with statistically significant associations with participant/maternal birth country per capita income ($p \leq 0.05$) are included in the figure. 'H' = High-income country, 'Um'= Upper-middle income country, and 'L' = Low-income country.

Maternal Education S.17

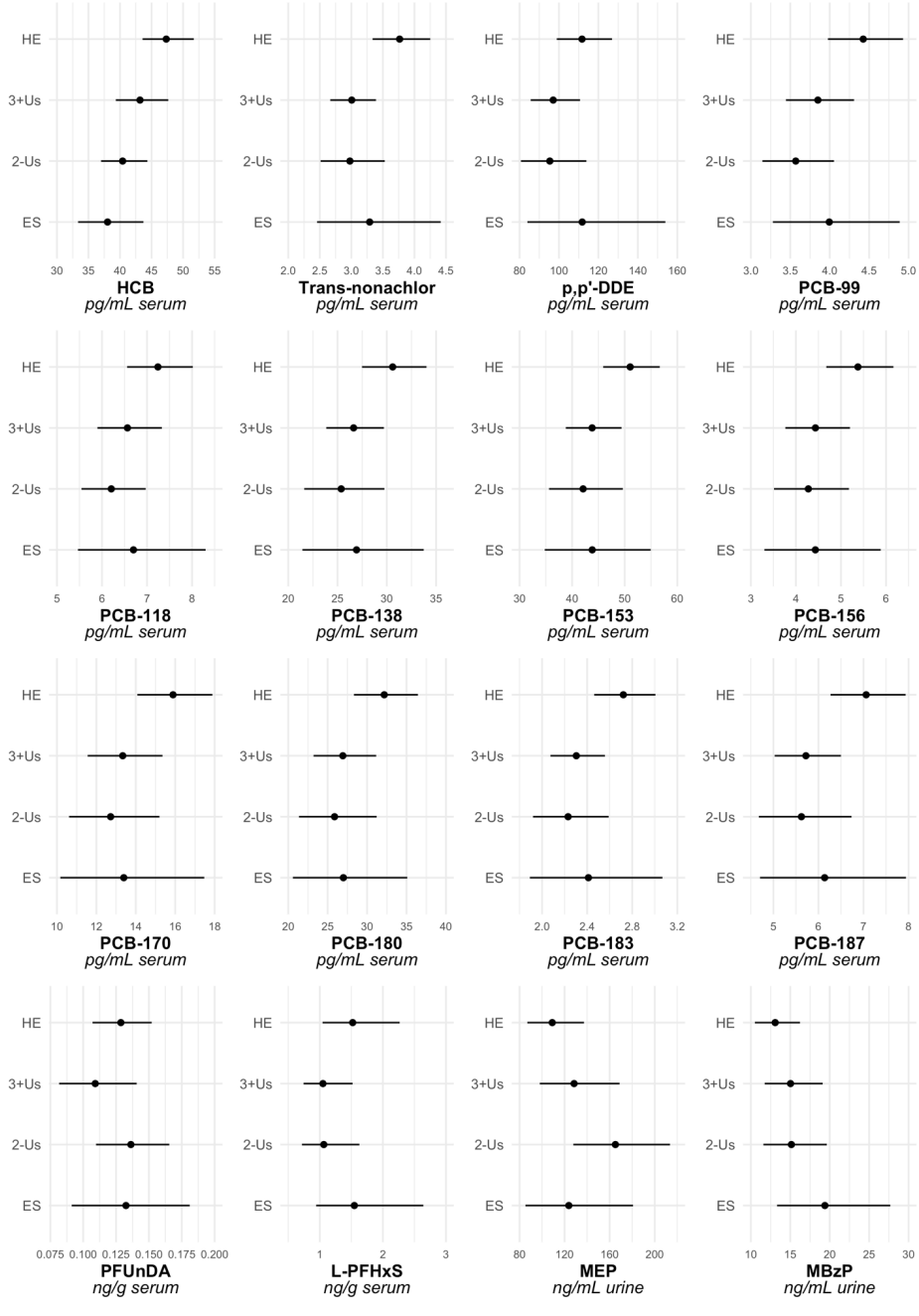

Figure 17: Associations between concentrations of substances given at the normal scale and the participants maternal education. The solid circle represents the estimated adjusted means concentration whilst the solid line represents the 95% confidence interval. Determined in ordinal regression analyses with participant age, gender, paternal education level, place of living (latitude and longitude) and participant/maternal birth country per capita income as covariates in the regression models. Only substances with statistically significant associations with maternal education ($p \leq 0.05$) are included in the figure. 'HE' = Higher education, '3+Us' = 3 years or more of upper-secondary, '2-Us' = 2 years or less of upper-secondary and 'ES' = 'Elementary school or none'.

Paternal Education S.18

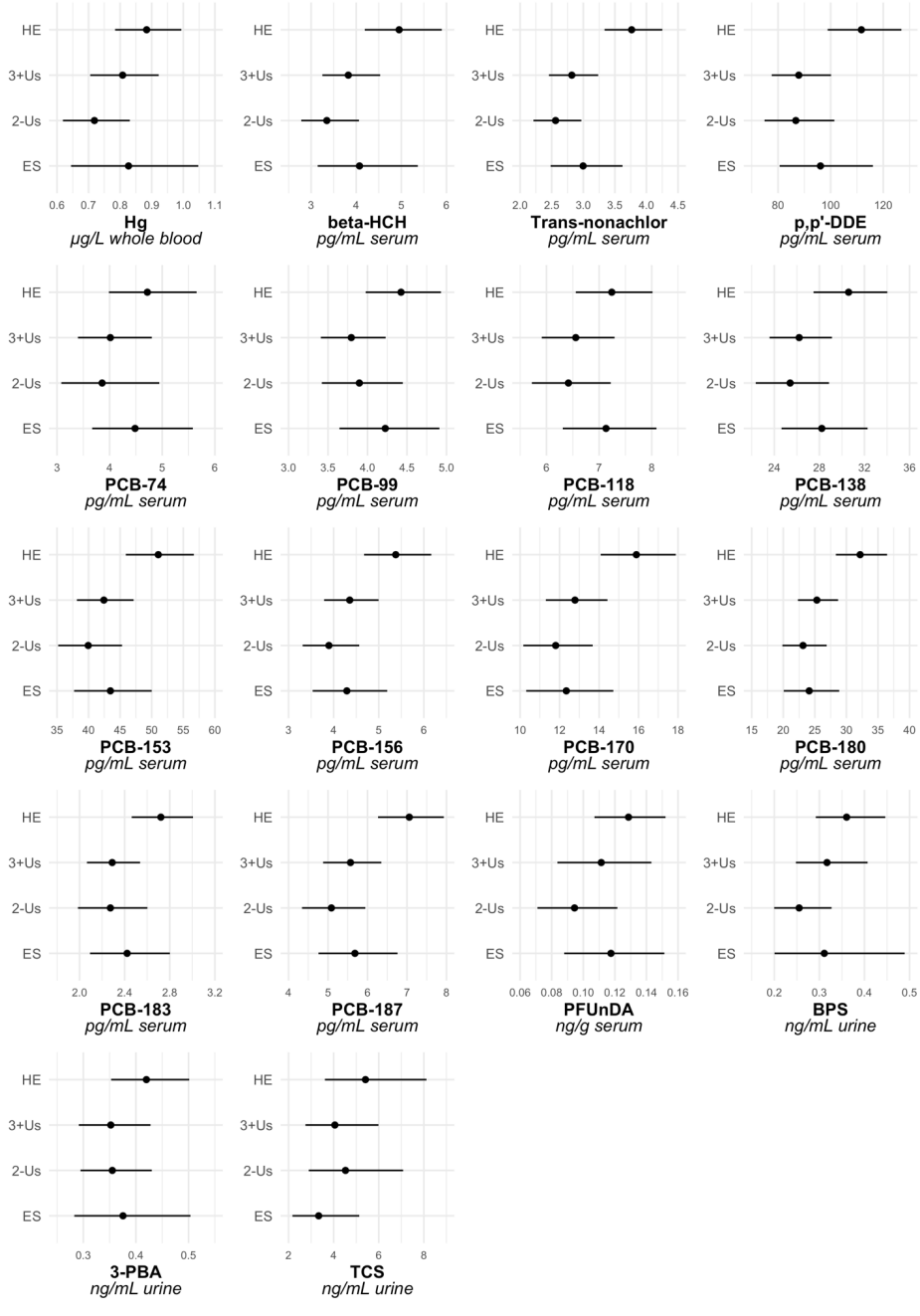

Figure 18: Associations between concentrations of substances given at the normal scale and the participants paternal education. The solid circle represents the estimated adjusted means concentration whilst the solid line represents the 95% confidence interval. Determined in ordinal regression analyses with participant age, gender, maternal education level, place of living (latitude and longitude) and participant/maternal birth country per capita income as covariates in the regression models. Only substances with statistically significant associations with paternal education ($p \leq 0.05$) are included in the figure. 'HE' = Higher education, '3+Us' = 3 years or more of upper-secondary, '2-Us' = 2 years or less of upper-secondary and 'ES' = 'Elementary school or none'.

Longitude S.19

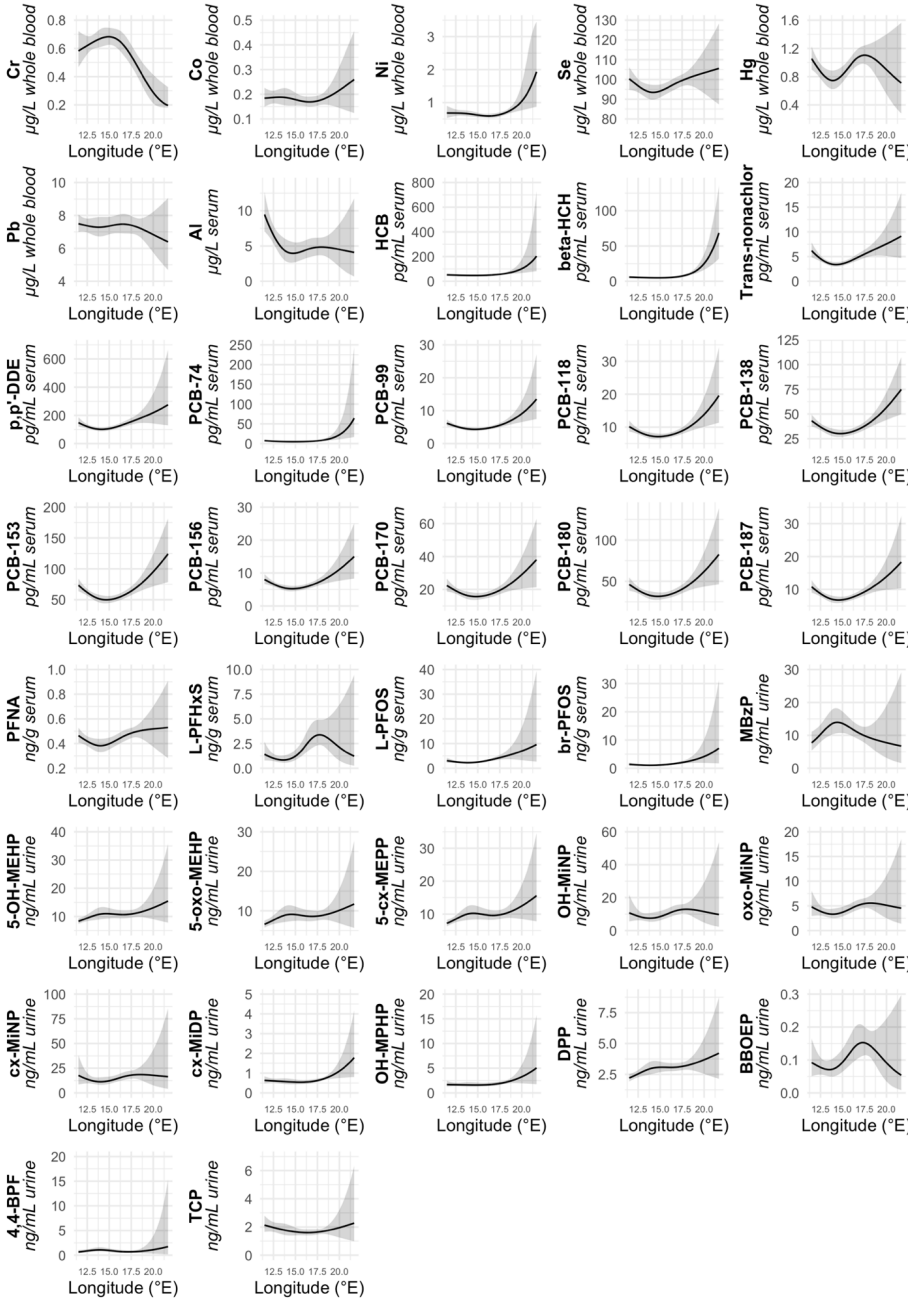

Figure S.19: Associations between concentrations of substances given at the normal scale and longitude of the home address. The solid line represents the estimated regression line whilst the shaded area represents the 95% confidence interval. Determined in ordinal regression analyses with participant age, gender, maternal and paternal education level, place of living (latitude) and participant/maternal birth country per capita income as covariates in the regression models. Only substances with statistically significant associations with longitude ($p \leq 0.05$) are included in the figure.

Additional covariate summary table

Table S.1; Summary statistics of additional covariates used only in the secondary models.

	Overall (N=1082)	Included in models for	References
Smoking			
N - Missing	20	Cd, Pb, PCBs, β-HCH, PFASs, 2-OH-PH, 1-HP	(Helmfrid et al., 2019; Lauritzen et al., 2016; Lignell et al., 2011; Shu et al., 2018; Wennberg et al., 2017; Willers et al., 1988)
No	939 (88.4%)		
Quit	23 (2.2%)		
Yes	100 (9.4%)		
Alcohol consumption			
N - Missing	23	Pb, PCBs, Trans-nonachlor	(Bjermo et al., 2013b; Lauritzen et al., 2016; Wennberg et al., 2017)
No	748 (70.6%)		
Yes, once	105 (9.9%)		
Yes, several times	206 (19.5%)		
Game meat products consumption			
N - Missing	1	Pb	(Bjermo et al., 2013b; Helmfrid et al., 2019; Wennberg et al., 2017)
Never	332 (30.7%)		
Seldom	265 (24.5%)		
Irregularly monthly	163 (15.1%)		
Often monthly	153 (14.2%)		
Irregularly weekly	96 (8.9%)		
Often weekly	72 (6.7%)		
Chocolate consumption			
N - Missing	7	PBA, MEHP, 5-OH-MEHP, 5-oxo-MEHP, 5-cx-MEHP, 2-cx-MEHP	(Larsson et al., 2014)
Never	42 (3.9%)		
Seldom	61 (5.7%)		
Irregularly monthly	63 (5.9%)		
Often monthly	154 (14.3%)		
Irregularly weekly	382 (35.5%)		
Often weekly	373 (34.7%)		
Ice cream consumption			
Never	51 (4.7%)	OH-MiNP, oxo-MiNP, cx-MiNP, cx-MiDP	(Larsson et al., 2014)
Seldom	227 (21.0%)		
Irregularly monthly	214 (19.8%)		
Often monthly	343 (31.7%)		
Irregularly weekly	168 (15.5%)		
Often weekly	79 (7.3%)		
Tube packaged foods consumption			
N - Missing	1	MEP	(Livsmedelsverkets, 2017)
Never	184 (17.0%)		
Seldom	267 (24.7%)		
Irregularly monthly	123 (11.4%)		
Often monthly	222 (20.5%)		
Irregularly weekly	152 (14.1%)		

Often weekly	133 (12.3%)		
Canned foods consumption			
N - Missing	126	BPA	(Covaci et al., 2015)
Never	361 (37.8%)		
Seldom	311 (32.5%)		
Monthly	219 (22.9%)		
Weekly	65 (6.8%)		
BMI (IOTF standard for children)			
Underweight	68 (6.3%)	PCBs, Trans-nonachlor, BDE-153	(Axmon et al., 2008; Glynn et al., 2007; Lauritzen et al., 2016; Lignell et al., 2011)
Normal weight	788 (72.8%)		
Overweight	185 (17.1%)		
Obese	41 (3.8%)		
Plasma ferritin levels (ug/L)			
N - Missing	22	Cd, Al	(Åkesson et al., 2011; Bjermo et al., 2013b; Julin et al., 2011; Nordberg and Nordberg, 2016)
Mean ± (SD)	37.9 (35.2)		
Median	31.0		
Range	1.7 - 797.0		
Egg consumption (g/day)			
Mean ± (SD)	22.5 (14.5)	PCBs, Trans-nonachlor, β-HCH, HCB, BDE-99	(Glynn et al., 2007; Lignell et al., 2011)
Median	18.7		
Range	2.0 - 95.1		
Meat consumption (g/day)			
Mean ± (SD)	126.6 (56.3)	BDE-153	(Bjermo et al., 2017)
Median	121.0		
Range	0.0 - 376.0		
Seafood consumption (g/day)			
Mean ± (SD)	23.0 (14.6)	Pb, Hg, PCBs, PFAS's Trans-nonachlor, β-HCH, DDE, HCB, BDE-153	(Bensryd et al., 1994; Bjermo et al., 2013b, 2013a; Glynn et al., 2007; Helmfrid et al., 2019; Lignell et al., 2011; Shu et al., 2018)
Median	20.8		
Range	0.0 - 101.0		
Season of sampling			
Autumn	492 (45.5%)	All secondary models	(Bastiaensen et al., 2021; Geller et al., 2023; Koppen et al., 2009; Makey et al., 2014)
Winter	186 (17.2%)		
Spring	404 (37.3%)		

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Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2024:90

This thesis analysed Swedish adolescents' exposure to chemical mixtures, including elements, persistent organic pollutants, organochloride pesticides, phthalates, phenols, and flame retardants, and its association with vaccination antibody levels. Relatively high percentage of adolescents exceeded recommended guidance values for some chemicals. Exposure levels varied by age, gender, and birth country, with the latter being most influential. Addressing exposure disparities is crucial for achieving the UN sustainability goals for good health and well-being, gender equality, and reducing inequalities within and amongst countries.

Sebastian Pineda received his doctoral education at the Department of Animal Biosciences, Swedish University of Agricultural Sciences. He graduated with a bachelor's degree in Microbiology from Nottingham Trent University and a master's degree in Infection Biology from Uppsala University.

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