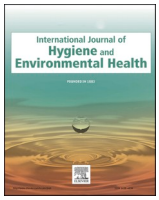
International Journal of Hygiene and Environmental Health 242 (2022) 113972

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh



Internal exposure of Flemish teenagers to environmental pollutants: Results of the Flemish Environment and Health Study 2016–2020 (FLEHS IV)

G. Schoeters [a,b,*], V.J. Verheyen [a,b], A. Colles [a], S. Remy [a], L. Rodriguez Martin [a], E. Govarts [a], V. Nelen [c], E. Den Hond [c], A. De Decker [c], C. Franken [c], I. Loots [d], D. Coertjens [d], B. Morrens [d], M. Bastiaensen [e], C. Gys [e], G. Malarvannan [e], A. Covaci [e], T. Nawrot [f], S. De Henauw [g], M. Bellemans [g], M. Leermakers [h], N. Van Larebeke [h,1], W. Baeyens [h], G. Jacobs [i], S. Voorspoels [i], F. Nielsen [j], L. Bruckers [k]

[a] VITO Health, Flemish Institute for Technological Research (VITO), Boeretang 200, 2400, Mol, Belgium
[b] Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium
[c] Provincial Institute of Hygiene, Kronenburgstraat 45, 2000, Antwerp, Belgium
[d] Department of Sociology, Faculty of Social Sciences, University of Antwerp, Sint-Jacobstraat 2, 2000, Antwerp, Belgium
[e] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium
[f] Centre for Environmental Sciences, Hasselt University, Agoralaan building D, 3590, Diepenbeek, Belgium
[g] Department of Public Health and Primary Care, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, 9000, Ghent, Belgium
[h] Analytical, Environmental and Geo- Chemistry, Vrije Universiteit Brussel, Brussels, Belgium
[i] VITO GOAL, Flemish Institute for Technological Research (VITO), Boeretang 200, 2400, Mol, Belgium
[j] Institute of Public Health, Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark
[k] BioStat, Data Science Institute, Hasselt University, Martelarenlaan 42, 3500, Hasselt, Belgium

ARTICLE INFO

Keywords:
Per-and polyfluoroalkyl substances
Plasticizers
Persistent halogenated compounds
Pesticides
Teenagers
Exposure biomarkers

ABSTRACT

The Flemish Environment and Health Study (FLEHS) collects information on internal exposure to a broad range of environmental chemicals in the general population in Flanders, the Northern region of Belgium. The aim is to establish biomonitoring exposure distributions for the general population in support of public health and environmental policy, environmental risk assessment and risk management decisions. In 2017–2018, urine and blood samples were collected from 428 teenagers by a stratified clustered two stage randomized design. Samples were analyzed for a broad range of biomarkers related to exposure to chlorinated and newer pesticides, brominated and organophosphate flame retardants (BFR/OPFR), polychlorinated biphenyls (PCBs), bisphenols, phthalates and alternative plasticizers, per-and polyfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAHs), benzene, metals and trace elements. The geometric mean levels and percentiles of the distribution were estimated for each biomarker, for the whole study population and following stratification for sex, the household educational attainment and the residence area's urbanicity.

Geometric means of biomarkers of lead, dichlorodiphenyltrichloroethane (DDT), PCBs, PAHs, regulated phthalates and bisphenol A (BPA) were lower than in the previous FLEHS cycles.

Most biomarker levels were below health-based guidance values (HB-GVs). However, HB-GVs of urinary arsenic, blood lead, blood cadmium, sum of serum perfluorooctane sulfonate (PFOS) and perfluoro-1-hexanesulfonate (PFHxS) and the urinary pyrethroid metabolite (3-PBA) were exceeded in respectively 25%, 12%, 39.5%, 10% and 22% of the teenagers. These results suggest that the levels of exposure in the Flemish population to some environmental chemicals might be of concern.

At the same time, we noticed that biomarkers for BPA substitutes, metabolites of OPFRs, an expanded list of PFAS, glyphosate and its metabolite could be measured in substantial proportions of participants. Interpretation of these levels in a health-risk context remains uncertain as HB-GVs are lacking.

Household educational attainment and residential urbanicity were significant exposure determinants for many biomarkers and could influence specific biomarker levels up to 70% as shown by multiple regression analysis.

* Corresponding author. University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, 2610, Wilrijk, Belgium.
E-mail address: greet.schoeters@uantwerpen.be (G. Schoeters).
[1] Study Centre for Carcinogenesis and Primary Prevention of Cancer, Ghent University Hospital, De Pintelaan 185, 9000, Ghent, Belgium

https://doi.org/10.1016/j.ijheh.2022.113972
Received 4 January 2022; Received in revised form 7 April 2022; Accepted 9 April 2022
Available online 19 April 2022

The research consortium also took care of the broader external communication of results with participants, policy makers, professional groups and civil society organizations. Our study demonstrated that teenagers are exposed to a wide range of chemicals, it demonstrates the success of public policies to reduce exposure but also points to concern and further priorities and needs for follow up.

Abbreviations

1-OH PYR	1- Hydroxypyrene
2,4-D	2,4-Dichlorophenoxyacetic acid
3-PBA	3-Phenoxybenzoic acid
AML	Algemeen Medisch Laboratorium
AMPA	Aminomethylphosphonic acid
ANOVA	Analysis of Variance
As	Arsenic
BBOEHEP	2-Hydroxyethyl bis(2-butoxyethyl) phosphate
BBzP	Benzyl butyl phthalate
B-Cd	Blood cadmium
BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate
BE	Biomonitoring Equivalent
BFR	Brominated flame retardants
BMI	Body Mass Index
BPA	Bisphenol A
B-Pb	Blood lead
BPF	Bisphenol F
BPS	Bisphenol S
CAS	Chemical Abstracts Service
Cd	Cadmium
CHOL	Cholesterol
CI	Confidence Interval
Cr	Creatinine
cx-MEPP	Mono-(2-ethyl-5-carboxypentyl) phthalate
DDE	Dichloro-diphenyl-dichloroethylene
DDT	Dichloro-diphenyl-trichloroethane
DEHA	Di-(2-ethylhexyl) adipate
DEHP	Bis(2-ethylhexyl) phthalate
DEHTP	Di-(2-ethylhexyl) terephthalate
DEP	Diethyl phthalate
DiBP	Diisobutyl phthalate
DINCH	Di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate
DnBP	Dibutyl phthalate
ECHA	European Chemicals Agency
EFSA	European Food Safety Agency
EHPHP	2-Ethylhexyl phenyl phosphate
eNO	Exhaled nitrogen oxide
ETS	Environmental Tobacco Smoke
EU	European Union
FAOSTAT	Food and Agriculture Organization of the United Nations
FLEHS	Flemish Environment and Health Study
FLU	Fluorene
GDPR	General Data Protection Regulation
GerES	German Environmental Survey
γ-HCH	Gamma-hexachlorocyclohexane
GLY	Glyphosate
GM	Geometric Mean
HB-GVs	Health-based guidance values
HBM	Human biomonitoring
HBM I	HBM-I value
HBM II	HBM-II value
HCB	Hexachlorobenzene
HCH	Hexachlorohexane
IARC	International Agency for Research on Cancer
ISCED	International Standard Classification of Education
LOD	Limit of Detection
LOQ	Limit of Quantification
MEHP	Mono-(2-ethylhexyl) phthalate
MEP	Monoethyl phthalate
MiBP	Mono-isobutyl phthalate
Mn	Manganese
MnBP	Mono-n-butyl phthalate
n	Sample size
NAPH	Naphthalene
NES	Neurobehavioral Evaluation System
NUTS	Nomenclature of Territorial Units for Statistics
OH	Hydroxy
OPFR	Organophosphate flame retardants
OXC	Oxychlordane
P50	50th Percentile
P90	90th Percentile
P95	95th percentile
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PBDEs	Polybrominated diphenylethers
PCBs	Polychlorinated biphenyls
PFAS	Per-and polyfluoroalkyl substances
PFDA	Perfluoro-n-decanoic acid
PFHxS	Perfluoro-1-hexanesulfonate
PFNA	Perfluorononan-1-oic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PHE	Phenanthrene
PIH	Provincial Institute of Hygiene of Antwerp
RQ	Risk Quotients
S	Serum
SD	Standard deviation
SES	Socio-economic status
SG	Specific Gravity
β-HCH	Beta-hexachlorocyclohexane
t,t'-MA	t,t'-Muconic acid
TCPγ	3,5,6-Trichloro-2-pyridinol
TG	Triglycerides
TI	Thallium
TN	Trans-nonachlor
TRA	Toxic relevant arsenic
U	Urinary
US-CDC	US-Centers for Disease Control and Prevention

1. Introduction

Environmental health concerns and a decree of Preventive Health Care voted in 2003 in Flanders, the Northern region of Belgium, led to the mandatory set up of a network for surveillance of human exposure and/or effects of exposure to physical and chemical factors in the population, intending to take measures to protect public health. As a follow-up, every five years since 2002, a cross-sectional cohort was established to assess the complex relationship between environmental pollution and

health. The Flemish Environment and Health Study (FLEHS) recruits a sample of a selected age group that is representative of sex and geographical area. Targets are set to include participants from different socio-economic statuses and the degree of urbanization of their residential neighborhood. FLEHS periodically measures concentrations of environmental chemicals and/or their metabolites in blood and urine of the study participants and relates it to their personal environment and life style (Schoeters et al., 2012). These exposure biomarkers, measured in easily accessible body fluids or tissues, aggregate chemical uptake from different sources and exposure routes and reflect the internal dose of a chemical (Angerer et al., 2007). In the fourth FLEHS campaign, FLEHS IV (2016–2020), 80 exposure biomarkers, belonging to eight different chemical classes, were measured including regulated compounds and substitutes that replace regulated and banned compounds.

Teenagers of 14 and 15 years old were included in FLEHS IV. They are considered sentinels of their local living environment and have been included in each of the FLEHS cycles. Teenagers may be particularly vulnerable to environmental exposures. The biology of adolescence is distinctive and provides opportunities for unique actions of toxicants both in terms of disruption of function and disruption of maturation (Golub, 2000). Getting adolescents involved in a human biomonitoring (HBM) study raises awareness of lifestyle and environment and how this may connect to exposure to hazardous chemicals and their health.

Exposure biomarkers were selected for chemical substances known to be toxic to humans (in particular carcinogenic or endocrine disruptor effects) and present in the environment of the Flemish population. We included biomarkers for chemicals that are associated with the residential neighborhood's spatial planning (metals, combustion-related compounds, pesticides), dietary habits (metals, persistent organic compounds, pesticides, plasticizers, per-and polyfluoroalkyl substances) and housing (combustion-related compounds, flame retardants, plasticizers). We also included biomarkers that were measured in previous HBM cycles, allowing to evaluate changes over time. For some of these substances, regulation at the European Union (EU) level and the member states exists. The efficiency of the regulation to decrease human exposure can be checked by HBM. As regulated substances are often replaced by other substances for which information on exposure and health effects is lacking, we also included biomarkers for chemicals of emerging concern.

This manuscript provides an overview of the internal exposure distributions of biomarkers in Flemish teenagers and puts them in a health risk context if health-based guidance values (HB-GVs) are available (Wilhelm, 2014). HB-GVs are considered as biomonitoring concentrations that are linked by physiology based pharmacokinetic modelling to chemical-specific intake limits. They correspond to binding effect levels derived from experimental animal studies or directly from human data based on a relationship between internal concentrations and health effects (Apel et al., 2020a). We compared the recent measurements in FLEHS IV with the levels measured in the previous cycles (Schoeters et al., 2017). Furthermore, we tested whether internal exposure to environmental chemicals was different depending on the urbanicity of the residence area and on the household educational attainment, as a proxy marker for socio-economic status (SES). Next to that, insight is given in the way these results were reported back to the participants and to the community they belong to.

2. Materials and methods

2.1. Study design and sampling

Four hundred twenty-eight teenagers aged 14 and 15 years old, took part in the FLEHS IV study. The sampling was organized between September 2017 and June 2018 according to a stratified clustered two stage sampling design. Within the age group, the study sample was representative of geographical location and sex. The first stratification corresponded with the Flemish provinces which are considered as level 2 according to the nomenclature of territorial units for statistics (NUTS) in the EU. The number of participants per province was proportional to the number of inhabitants per province. Primary sampling units were schools that were randomly selected in each province. Distances between schools had to be at least 20 km and one school in the highest quartile of socially deprived attendants was included in each province to ensure participation of all socio-economic categories. Teenagers and one of the parents had to give their signed informed consent. Further inclusion criteria were: living in Flanders for at least five years and teenagers and parents mastered enough Dutch to fill out extensive questionnaires. Exclusion criteria were: more than one questionnaire not filled out, blood and urine sample missing, being held back in school for more than one year, attending a boarding school. Data of one subject were not considered in the analysis due to pregnancy. The FLEHS IV study protocol was approved in June 2017 by the Antwerp University Hospital Ethics committee (Belgian registration number B300201732753). Included as well was a statement on the report-back of the individual exposure results to the parents and teenager, and if preferred to their general practitioner. The medical practitioner of the Provincial Institute of Hygiene (PIH) also intercepted individual questions of respondents on their results afterwards.

Before clinical examination, teenagers filled out extensive questionnaires on health status and life style patterns such as the use of cosmetics, tobacco and alcohol, dietary habits, time spent in traffic, and their opinion on their environmental risk perception, attitudes and eco-behavior. Additionally, a short questionnaire was filled out at the day of sampling on recent exposure (i.e. within the last three days), e.g. smoking, medication, alcohol and food consumption. Parents filled out a questionnaire on the home environment, the housing conditions, pregnancy of the data subject, SES (e.g. educational level of the parents, household income). Teenagers and parents together filled out the food questionnaire. The participants chose to use electronic or paper questionnaires; the latter was preferred by 60% of parents and 40% of teenagers. The main characteristics of the study participants are presented in Table 1 and compared to information of the general Flemish population of the same age group to check representativity of the FLEHS IV study participants.

Clinical examinations were organized at the school location, they took about 1 h and were performed by trained nurses. The teenagers donated a urine sample of minimally 46 mL, a hair sample of 3 cm that was taken close to the scalp and a 35 mL blood sample. Urine samples were collected in clean metal-free polyethylene containers; they were kept at 4 °C and processed within 24 h. Samples were divided into aliquots with glass vials as recipients for measurement of the biomarkers for plastic compounds, polypropylene tubes for measurement of the biomarkers for benzene, PAHs and arsenic species. Metal-free polyethylene tubes were used for measurement of the other metal biomarkers. Until analysis, all samples were kept at −20 °C, except the vials for measurement of biomarkers of benzene and PAHs that were kept at −80 °C. The blood samples were immediately processed: 2 aliquots of whole blood and serum were kept at 4 °C and stored at −20 °C or −80 °C within 12 h in a central lab. When field work was finished, all samples, together with field work blanks and control samples, were shipped on dry ice to the analytical laboratories until sample work-up.

Clinical examinations included measuring body height and body weight while teenagers were not wearing shoes but were fully clothed. Abdominal circumference provided information on obesity risk. Blood pressure was measured according to the European Society of Hypertension guidelines using an automated blood pressure instrument. Exhaled nitrogen oxide (eNO) was measured as an indicator for airway inflammation. A Neurobehavioral Evaluation System computerized battery of tests (NES) was used to assess sustained attention, short-term memory and manual motor speed. Sustained attention was assessed by continuous performance and Stroop test, the short-term memory by the digit span forward and backward and the motor speed by a finger tapping task (Kicinski et al., 2016). Collection, storage, transfer, and use of

Table 1
Main characteristics of teenagers (14–15 years) selected in the FLEHS IV study in comparison with the Flemish population at age 15. Sample size and % of the study population.

	FLEHS IV	Flemish reference population 15 years
Sex		Statbel 2018 [1]
Boys	199 (46.5%)	34502 (51.3%)
Girls	229 (53.5%)	32795 (48.7%)
Age (years)		
≤14.5	117 (27.3%)	
14.5–15.5	277 (64.7%)	
>15.5	34 (7.9%)	
Educational level		Number of students in the third year of secondary education in schools of the Flemish region, 2017–18[2]
Vocational education	79 (18.5%)	20.6%
Technical education	133 (31.1%)	27.9%
General education	216 (50.5%)	49.6%
Art education	0 (0%)	1.9%
Household educational attainment (ISCED)		Statbel 2018 [3]
Primary	26 (6.2%)	19%
Secundary	140 (33.4%)	40%
Tertiary	254 (60.4%)	41%
Country of birth		Flemish region, 2016 [4]
Non-EU	43 (10.1%)	11.3%
EU	36 (8.4%)	9.2%
Belgium	348 (81.5%)	79.5%
Urbanicity (Eurostat)		Eurostat 2016
Rural areas	59 (13.8%)	9%
Towns and suburbs	311 (72.7%)	72%
Cities	58 (13.6%)	19%
Province of residence		Statbel, 2018 [1]
Antwerp	117 (27.3%)	28.2%
East-Flanders	91 (21.3%)	23.0%
West-Flanders	82 (19.2%)	18.2%
Limburg	62 (14.5%)	13.3%
Flemish-Brabant	76 (17.8%)	17.4%
Season		
Autumn	100 (23%)	
Winter	138 (32%)	
Spring	190 (44%)	
Summer	0 (0%)	
Smoking behavior		Student survey, 2016–2017 [5]
Never or once	409 (95.8%)	93.1%
Seldom	8 (1.9%)	4.1%
Daily	10 (2.3%)	2.7%
Exposure to environmental tobacco smoke		
Yes	161 (38.2%)	
No	261 (61.9%)	
Alcohol use		Student survey 2016–2017 [5]
Never or very seldom	365 (85.7%)	68.2%
< weekly	52 (12.2%)	23.4%
Weekly	9 (2.1%)	8.4%
Body Mass Index boys [6]		Food consumption survey 2014 [7]
Underweight	17 (8.5%)	10.0%
Normal weight	152 (76.4%)	77.6%
Overweight, obese	30 (15.1%)	12.5%
Body mass Index girls [6]		Food consumption survey 2014 [7]
Underweight	18 (7.9%)	10.7%
Normal weight	156 (68.1%)	69.0%
Overweight, obese	55 (24.0%)	20.4%

[1] https://Statbel.fgov.be/.
[2] http://www.ond.vlaanderen.be/.
[3] https://Statbel.fgov.be/; Highest educational level of Flemish adults between 25 and 64 years (n = 3 445 221) with BSO: Vocational Secondary Education, TSO: Technical Secondary Education, ASO: General Secondary Education, KSO: Artistic Secondary Education.
[4] Flemish Regional Indicators (VRIND) 2017; https://www.vlaanderen.be/publicaties/vrind-2017-vlaamse-regionale-indicatoren.
[5] Flemish Expertise Centre Alcohol and drugs: questioning of students 13-16 years- (2016–2017), https://www.vad.be/.
[6] Flemish growth curves of 2004, https://www.vub.be/groeicurven/.
[7] Sciensano 30/08/2019, information from Flemish students (10–17 years); International Obesity Task Force Criteria used as cut off points.

data were carried out according to the European General Data Protection Regulation (GDPR, Regulation (EU) 2016/679). The database was registered at the Belgian Committee for Privacy Protection (VT005081316). All data were pseudonymized prior to further analysis. According to the communication strategy and its agreed guiding principles, the participants received their interpretable personal results, positioned against the median and 90th percentile of the total study group and compared to the thresholds (HB-GVs). They also received a summary of the aggregated study results; background information on the chemical substances and suggestions for health promotional measures. The generic information is also made available on the website https://www.milieu-en-gezondheid.be/, including short video presentations. It is the consortium's broad interdisciplinary configuration - including toxicologists, biologists, medical scientists, statisticians and social scientists - that ensures the combination of these different components in the study design.

2.2. Selection and analysis of exposure biomarkers

Arsenic (As), cadmium (Cd), lead (Pb) and thallium (Tl) were included as metals. Biomarkers for these compounds have been measured in previous cycles.

PAHs and benzene were selected as combustion-related compounds. We have biomonitoring data from previous FLEHS studies for the pyrene metabolite 1- hydroxypyrene (1-OH PYR) and for t,t'-muconic acid (t,t'-MA) as benzene metabolite. In the present study, we included additional metabolites for some more volatile PAHs: naphthalene (NAPH), phenanthrene (PHE) and fluorene (FLU).

Plastic compounds included bisphenols and metabolites of phthalates that were measured in the urine samples.

To assess exposure to pesticides, biomarkers for pyrethroids, chlorpyrifos, the phenoxyherbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and glyphosate (GLY) and its metabolite aminomethylphosphonic acid

(AMPA) were measured for the first time in Flemish teenagers. Legacy pesticides, such as beta-and gamma-hexachlorocyclohexane (β-HCH and γ-HCH), p,p'-dichloro-diphenyl-trichloroethane (DDT) and its metabolite p,p'-dichloro-diphenyl-trichloroethane (DDE), the chlordane-related compounds oxychlordane (OXC) and trans-nonachlor (TN) and the fungicide hexachlorobenzene (HCB) were measured in serum samples in FLEHS IV and in previous studies.

Flame retardants measured in serum included the polybrominated diphenylethers (PBDEs), while metabolites of organophosphate flame retardants (OPFRs), that are replacing the PBDEs, were measured in urine. Other persistent substances that were measured are marker PCBs (PCB138, PCB153 and PCB180) and PFAS. Marker PCBs were also measured in teenagers in all three previous FLEHS studies. We measured for the first time in serum of teenagers, recruited from the general population, twelve different long-chain perfluoroalkyl substances, including perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononan-1-oic acid (PFNA) and the slightly shorter perfluorohexane-1-sulphonic acid (PFHXS) (Supplemental Material S 1).

Table 2 lists the targeted parent compounds, the biomarkers that were selected, the analytical methods and the references to publications that report on the treatment of samples, analytical methods and quality assurance protocols. Table 2 also lists the Limit of Detection (LOD) or Limit of Quantification (LOQ) as provided by the analytical laboratories. Information on linearity, reproducibility, repeatability, accuracy, precision and quality assurance procedures of each of the analytical methods was collected. Triglycerides (TG) and cholesterol (CHOL) were routinely measured in blood samples. The following formula calculated total blood lipid concentration- TL = 1.33*TG + 1.12*CHOL*148 (g/L) (Covaci and Voorspoels, 2005) and was used to standardize lipid-soluble serum biomarkers. Specific gravity (SG) and creatinine (Cr) were determined in urine by refractometry and the Jaffe method at the General Medical Laboratory (Algemeen Medisch Laboratorium -AML, Antwerp, Belgium). Urinary biomarker concentrations were normalized for SG using the following formula: $biomarker_{SG} = C_{biomarker} * (1.024\text{-}1)/(SG\text{-}1)$ with $biomarker_{SG}$ as the normalized biomarker concentration, $C_{biomarker}$ as the measured biomarker concentration per liter urine and SG as the specific gravity of the urine sample (Pearson et al., 2009). Urinary dilution was checked based on criteria set for unsubstituted urine samples (Barbanel et al., 2002). All urine samples had SG above 1.001 and Cr above 5 μg/dL.

2.3. Statistical analysis

Detection frequencies were calculated for each chemical biomarker. Statistical analyses were carried out if biomarker values were above LOQ in at least 60% of the samples. Values below the LOQ were imputed with a random value (between 0 and the LOQ), drawn from the estimation of the lognormal distribution of all values by fitting a truncated lognormal distribution using only values above the LOQ (Lubin et al., 2004). The median, 90th and 95th percentile (P90, P95) and the geometric mean (GM) with 95% confidence interval (95% CI) were calculated. The CI for the percentiles are distribution-free using order statistics (Hahn et al., 2016). PCBs are reported as the sum of the marker PCBs (PCB138, PCB153, PCB180).

The proportion of participants with values above HB-GVs was calculated for biomarkers for which HB-GVs were available. We selected HB-GVs derived by international organizations such as the European Chemicals Agency (ECHA) or the European Food Safety Agency (EFSA), or published in peer-reviewed literature with preference to more recently derived values (Table 3). In addition, risk quotients (RQ) were calculated as the ratio of the biomarker concentration, at the GM or P95, to the chemical-specific HB-GV: RQ = [biomarker]/HB-GV).

Several biomarkers have been measured in the same age group in previous FLEHS studies, starting from 2002. Comparisons in time until FLEHS III have been published earlier (Schoeters et al., 2017). To allow comparison with previous FLEHS cycles, biomarkers were treated in the same way as in previous cycles. Values below LOD/LOQ were imputed by half LOD/LOQ. To check analytical comparability, biobank samples from previous FLEHS cycles were included in the present analytical runs. Multiple regression models were established based on the pooled dataset of the different cycles to evaluate the influence of time on chemical biomarker concentrations. To account for differences in composition of the sampled study participants between the successive cycles, all models were adjusted for sex, smoking, age and body mass index (BMI). Additionally, urinary biomarkers were adjusted for Cr and lipid soluble serum biomarkers were adjusted for blood lipids. All these variables were categorized (Table 1). Analysis of variance (ANOVA) was used to test for differences among biomarker-specific GMs of different FLEHS studies. If differences were statistically significant ($p < 0.05$), a two sided t -test was used to assess the difference of the GM of the FLEHS IV HBM data with those of previous studies. This allows complementing the time trend analysis (Schoeters et al., 2017) with the new data of the FLEHS IV study.

We also tested whether neighborhood urbanicity and SES of the household influenced the exposure biomarkers. Urinary biomarkers were normalized to SG, lipid soluble serum biomarkers were normalized to serum lipids. Participants' home addresses were geocoded. The Eurostat definition of urbanicity was used to classify the degree of urbanization of the residence area. The Eurostat definition is based on a combination of geographical contiguity and population density, applied to 1 km^2 population grid cells (Eurostat, 2018). Eurostat defines neighborhoods as 1) cities: densely populated areas where 50% or more of the population lives in urban centers with a population density of at least 1500 inhabitants per km^2 and at least 50 000 inhabitants, 2) towns/suburbs: intermediate density areas where 50% or more of the population lives in urban clusters with a population density of at least 300 inhabitants per km^2 and a minimum population of 5000 inhabitants, 3) rural areas: sparsely populated areas outside of city centers and urban clusters. The variable could be calculated for all participants.

The highest household educational attainment was taken as a proxy for the SES of the household. The information was available for 420 out of 428 participants. Individuals with missing values were not included in the analysis. Three categories were considered based on to the Belgian education system: primary (no educational attainment, primary school, lower secondary school), secondary (higher secondary school) and tertiary (higher education attainment). These levels correspond to the codes 0–2, 3–4 and 5–8 of the International Standard Classification of Education (ISCED). To test the influence of neighborhood urbanicity and SES of the household on the exposure biomarkers, multiple regression models were built. The exposure markers were used as dependent variables. They were log-transformed to achieve normal distributions. A first model was built that included the variables of interest, either urbanicity or educational attainment, as well as variables that may influence the exposure biomarkers but are known not to be associated with the variables of interest such as sex, age, sampling season and additionally serum ferritin for metals, SG for urinary markers, blood lipids for lipid-soluble serum markers. Serum ferritin, SG and blood lipid concentrations were used as continuous variables and other variables were categorized. We assessed whether the variable of interest was significant in these models. If significant ($p < 0.05$) or borderline significant ($p < 0.10$), the estimated biomarker levels were compared with the reference categories which were the primary educational level for the models with the household educational attainment as the variable of interest and with cities for the degree of urbanicity as a variable of interest. Further models included variables that may influence the exposure biomarkers and may be linked to the variable of interest such as smoking behavior, BMI, being breastfed. Boys and girls were classified as underweight, average weight, overweight or obese according to the sex- and age-specific 2004 Belgian growth curves (Roelants et al., 2009). Finally, we tested whether differences in biomarker levels between girls and boys remained significant in a multivariate model after adjustment for the above-mentioned variables, household educational attainment

Table 2
Biomarker levels measured in FLEHS IV teenagers normalized to specific gravity (urinary markers) and serum lipids (lipid-soluble serum biomarkers).

Parent compound (CAS nr)	Biomarker	Analytical Method	Unit	LOD/ LOQ	Sample size	% above LOD/ LOQ	GM (95% CI)	P50	P90	P95 (95%CI)
Metals and trace elements in urine										
Anorganic arsenic7440-38-2	Arsenobetaïne	UPLC-MS/MS(De Craemer et al., 2017)	µg/ L	LOD = 0.1	194	83.5	1.44 (0.96;2.15)	2.17	30.1	46.8 (34.8;78.3)
	Arsene(III)			LOD = 0.1	194	70.6	0.18 (0.14;0.23)	0.32	0.88	1.19 (0.98;1.80)
	Arsene(V)			LOD = 0.1	194	26.3	NC	<LOD	0.28	0.36 (0.29;0.58)
	Mono methyl arsenate (MMA)			LOD = 0.1	194	83.0	0.35 (0.28;0.45)	0.60	1.29	1.66 (1.44;2.16)
	Dimethyl arsenate (DMA)			LOD = 0.1	194	100	3.59 (3.29;3.91)	3.28	8.07	11.62 (9.66;14.58)
	Toxic relevant arsenic (TRA)(sum As III, As V, DMA, MMA)			NA	194		4.62 (4.26;5.02)	4.23	10.30	14.16 (11.48;16.52)
Cadmium7440-43-9	Cadmium (U-Cd)	HR-ICP-MS (Schroijen et al., 2008)		LOD = 0.010	415	100	0.300 (0.287;0.313)	0.287	0.494	0.630 (0.545;0.850)
Thallium 7440-28-0	Thallium (U-Tl)			LOD = 0.002	415	100	0.355 (0.345;0.365)	0.359	0.502	0.562 (0.539;0.582)
Metals and trace elements in blood										
Cadmium7440-43-9	Cadmium (B-Cd)	HR-ICP-MS (Schroijen et al., 2008)	µg/ L	LOD = 0.007	419	100	0.19 (0.18;0.20)	0.18	0.29	0.38 (0.34;0.51)
Thallium 7440-28-0	Thallium (B-Tl)		ng/ L	LOD = 0.651	419	100	27.3 (26.7;27.9)	26.8	35.6	38.9 (37.3;42.0)
Lead7439-92-1	Lead (B-Pb)		µg/ L	LOD = 0.048	419	100	7.7 (7.4;8.0)	7.7	12.8	14.3 (13.6;17.5)
Manganese7439-96-5	Manganese (B-Mn)		µg/ L	LOD = 0.120	419	100	9.4 (9.1;9.6)	9.3	13.7	15.6 (14.6;16.2)
Copper7440-50-8	Copper (B-Cu)		µg/ L	LOD = 0.551	419	100	816 (801;831)	792	1024	1252 (1093;1378)
Zinc7440-66-6	Zinc (B-Zn)		mg/ L	LOD = 0.008	419	100	5.29 (5.21;5.37)	5.35	6.53	6.88 (6.72;7.08)
Polycyclic aromatic hydrocarbons and benzene in urine										
Pyrene129-00-0	1-Hydroxypyrene (1-OH PYR)	UPLC-MS/MS(Verheyen et al., 2021)		LOQ = 0.015	412	97.6	0.067 (0.063;0.071)	0.066	0.134	0.164 (0.142;0.218)
Naphthalene91-20-3	2-Hydroxynaphthtalene (2-OH NAPH)			LOQ = 0.150	413	100	4.07 (3.73;4.43)	3.75	12.39	19.4 (15.1;24.5)
Fluorene86-73-7	sum of 2- and 3-Hydroxy-fluorene (2-OH FLU + 3-OH FLU))			LOQ = 0.030	413	99.5	0.208 (0.197;.0.220)	0.192	0.403	0.555 (0.466;0.689)
Phenanthrene85-01-8	2-Hydroxyphenantrene (2-OH PHE)			LOQ = 0.015	414	97.6	0.074 (0.070;0.078)	0.072	0.147	0.178 (0.162;0.205)
	3-Hydroxyphenantrene (3-OH PHE)			LOQ = 0.014	414	98.8	0.073 (0.069;0.077)	0.072	0.133	0.168 (0.142;0.219)
	sum of 1- and 9-Hydroxy-phenantrene (1-OH PHE + 9-0H PHE)			LOQ = 0.031	414	98.1	0.111 (0.104;0.118)	0.105	0.240	0.323 (0.266;0.546)
	4-Hydroxyphenantrene (4-OH PHE)			LOQ = 0.014	414	7.00	NC	<LOQ	<LOQ	0.018 (<LOQ;0.030)
Benzene71-43-2	T,t'-muco nic acid (t,t'-MA)	HPLC-UV (Schoeters et al., 2017)		LOD = 2	415	99.3	92.0 (83.8;101.0)	101	251	422 (300;600)
Bisphenols in urine										
80-05-7	Bisphenol A (BPA)	GC-MS/MS (Gys et al., 2020)	µg/ L	LOQ = 0.3 µg/L	414	85.7	1.07 (0.98;1.18)	1.15	3.10	5.13 (3.71;5.85)
620-92-8	Bisphenol F (BPF)			LOQ =	414	96.6	0.17 (0.15;0.19)	0.15	0.73	1.12 (0.86;1.63)

(continued on next page)

Table 2 (*continued*)

Parent compound (CAS nr)	Biomarker	Analytical Method	Unit	LOD/ LOQ	Sample size	% above LOD/ LOQ	GM (95% CI)	P50	P90	P95 (95%CI)
80-09-1	Bisphenol S (BPS)			0.02 µg/L LOQ = 0.04 µg/L	414	83.3	0.13 (0.11;0.14)	0.14	0.46	0.83 (0.68;1.13)
77-40-7	Bisphenol B (BPB)			LOQ = 0.02 µg/L	414	57.0	NC	0.03	0.08	0.11 (0.10;0.13)
843-55-0	Bisphenol Z (BPZ)			LOQ = 0.03 µg/L	414	37.0	NC	<LOQ	0.08	0.18 (0.11;0.31)
1478-61-1	Bisphenol AF (BPAF)			LOQ = 0.02 µg/L	414	12.0	NC	<LOQ	0.02	0.03 (0.03;0.04)
Phthalates in urine										
Diethyl phthalate84-66-2	Monoethyl phthalate (MEP)	LC-MS/MS (Bastiaensen et al., 2021a)	µg/L	LOQ = 0.5	407	100	37.9 (33.3;43.2)	27.2	244.9	569 (346;1332)
Di-n-butyl phthalate84-74-2	Mono-n-butyl phthalate (MnBP)			LOQ = 0.5	407	100	19.7 (18.5;21.0)	19.2	44.8	56.8 (52.2;78.3)
Di-isobutyl phthalate84-69-5	Mono-isobutyl phthalate (MiBP)			LOQ = 0.5	407	100	25.5 (23.5;27.7)	22.5	70.9	120 (83.5;184)
Butylbenzyl phthalate85-68-7	Mono-benzyl phthalate (MBzP)			LOQ = 0.2	407	98.3	3.0 (2.7;3.4)	2.4	15.9	36.0 (27.8;43.5)
Di-2-ethylhexyl phthalate117-81-7	Mono-(2-ethylhexyl) phthalate (MEHP)			LOQ = 0.5	407	83.5	12.5 (11.7;13.4)	12.2	31.9	41.0 (35.9;53.3)
	Mono-(2-ethyl-5-oxohexyl) phthalate (5-oxo-MEHP)			LOQ = 0.2	407	99.5	4.2 (3.9;4.6)	4.1	11.0	15.1 (12.7;19.3)
	Mono-(2ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP)			LOQ = 0.2	407	99.7	6.7 (6.2;7.2)	6.6	17.5	21.5 (19.5;29.6)
	Mono-(2-ethyl-5-carboxypentyl) phthalate (5-c x-MEPP)			LOQ = 0.5	407	100	16.4 (15.7;17.1)	16.2	28.7	34.2 (31.7;41.4)
Pesticides in urine										
Pyrethroids8003-34-7	3-Phenoxybenzoic acid (3-PBA)	HPLC-TQMS (Andersen et al., 2021)	µg/L	LOD = 0.03	415	99.5	0.953 (0.870;1.044)	0.871	2.77	4.14 (3.09;5.75)
Chloropyrifos 2921-88-2	3,5,6-trichloro-2-pyridinol (TCPγ)			LOD = 0.3	415	98.5	4.25 (3.98;4.54)	4.45	10.1	12.3 (10.7;13.6)
Phenoxyherbicide94-75-7	2,4-dichlorophenoxyaceticacid (2,4-D)			LOD = 0.03	415	96.1	0.259 (0.238;0.282)	0.274	0.733	1.003 (0.830;1.135)
Glyphosate1071-83-6	Glyphosate (Gly)	GC-MS/MS (Lemke et al., 2021)	µg/L	LOQ = 0.1	415	41.4	NC	<LOQ	0.280	0.391 (0.332;0.468)
A-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid1066-51-9	Aminomethylphosphonic acid (AMPA)		µg/L	LOQ = 0.1	415	55.9	NC	0.11	0.284	0.371 (0.323;0.448)
Chlorinated pesticides in serum										
Hexachloro Benzene 118-74-1	Hexachlorobenzene (HCB)	GC-ECNI/MS (Covaci and Voorspoels, 2005)	ng/g lipid	LOQ = 2 ng/L	415	100	7.5 (7.2;7.8)	7.6	12.0	14.1 (12.7;15.2)
Dichlorodi Phenyltri Chloroethane 50-29-3	DDT			LOQ = 4 ng/L	415	74.5	1.8 (1.6;2.1)	2.4	8.1	16 (10.9;22.1)
	DDE			LOQ = 4 ng/L	415	100	40.9 (37.9;44.2)	34.4	123	193 (149;247)
Chlordane39765-80-5	Oxychlordane (OXC)			LOQ = 2 ng/L	415	95.4	1.21 (1.14;1.28)	1.21	2.39	3.38 (2.73;3.79)
	Trans-nonachlor (TN)			LOQ = 2 ng/L	415	85.3	0.77 (0.72;0.83)	0.80	1.71	2.37 (2.08;2.62)
Lindane58-89-9	Beta-hexachlorocyclohexane (β-HCH)			LOQ = 2 ng/L	415	95.9	1.13 (1.06;1.20)	1.12	2.15	3.01 (2.39;4.78)

(*continued on next page*)

Table 2 (*continued*)

Parent compound (CAS nr)	Biomarker	Analytical Method	Unit	LOD/ LOQ	Sample size	% above LOD/ LOQ	GM (95% CI)	P50	P90	P95 (95%CI)
	Gamma-hexachlorocyclohexane (γ-HCH)			LOQ = 2 ng/L	415	16.1	NC	<LOQ	<LOQ	0.80 (0.70;0.90)
Polychlorinated biphenyls in serum										
Polychlorinated biphenyls	Marker PCBs (sum of PCB 138, 153, 180)	GC-ECNI/MS (Covaci and Voorspoels, 2005)	ng/g lipid	LOQ = 2 ng/L	415	NA	21.6 (20.4;22.9)	20.8	47.8	57.1 (51.5;72.8)
Brominated Flame retardants in serum										
41318-75-6	BDE 28	GC-ECNI/MS (Covaci and Voorspoels, 2005)	ng/g lipid	LOQ = 1 ng/L	415	0.48	NC	<LOQ	<LOQ	<LOQ
5436-43-1	BDE 47			LOQ = 1 ng/L	415	53.6	NC	<LOQ	0.80	1.20 (1.00;1.60)
60348-60-9	BDE 99			LOQ = 1 ng/L	415	39.7	NC	<LOQ	0.80	1.00 (0.90;1.20)
32534-81-9	BDE 100			LOQ = 1 ng/L	415	0.72	NC	<LOQ	<LOQ	<LOQ
488710-22-1	BDE 153			LOQ = 2 ng/L	415	21.4	NC	<LOQ	0.70	0.90 (0.80;1.40)
207122-15-4	BDE 154			LOQ = 2 ng/L	415	37.5	NC	<LOQ	0.70	0.90 (0.80;0.90)
207122-16-5	BDE 183			LOQ = 2 ng/L	415	0.00	NC	<LOQ	<LOQ	<LOQ
Organophosphate flame retardants and plasticizers (PFRs) in urine										
Triphenylphosphate115-86-6	4-Hydroxyphenyl phenyl phosphate (4-OH-DPHP)	LC-MS/MS (Bastiaensen et al., 2018)	μg/L	LOQ = 0.5 μg/L	413	13.5	NC	<LOQ	0.72	1.05 (0.83;1.47)
	Diphenyl phosphate (DPHP)			LOQ = 0.1 μg/L	413	99.0	1.36 (1.27;1.46)	1.33	3.19	3.97 (3.49;4.47)
	Hydroxyphenyl diphenyl phosphate (OH-TPHP)			LOQ = 0.01 μg/L	413	5.69	NC	<LOQ	<LOQ	0.01 (<LOQ;0.01)
2-ethylhexyl diphenyl phosphate1241-94-7	2-Ethylhexyl phenyl phosphate (EHPHP)			LOQ = 0.05 μg/L	413	99.3	4.18 (3.89;4.50)	4.13	10.6	12.7 (11.6;15.7)
	2-Ethyl-5-hydroxyhexyl diphenyl phosphate (5-OH-EHDPHP)			LOQ = 0.01 μg/L	413	98.3	0.09 (0.08;0.10)	0.09	0.27	0.34 (0.30;0.41)
Tris(2-chloroisopropyl phosphate13674-84-5	1-Hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP)			LOQ = 0.04 μg/L	413	95.2	0.70 (0.61;0.80)	0.69	3.59	6.88 (4.78;14.34)
	Bis(1-chloro-2-propyl) phosphate (BCIPP)			LOQ = 1 μg/L	413	8.53	NC	<LOQ	<LOQ	2.93 (1.27;4.38)
Trischloroethylphosphate115-96-8	Tris(chloroethyl) phosphate(TCEP)			LOQ = 0.04 μg/L	413	28.7	NC	<LOQ	0.09	0.12 (0.10;0.15)
Tris(2-butoxyethyl) phosphate78-51-3	Bis(2-butoxyethyl) phosphate (BBOEP)			LOQ = 0.05 μg/L	413	10.2	NC	<LOQ	0.05	0.10 (0.08;0.13)
	2-Hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP)			LOQ = 0.005 μg/L	413	95.6	0.040 (0.037;0.045)	0.037	0.129	0.245 (0.147;0.320)
	Bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate (3-OH-TBOEP)			LOQ =	413	5.81	NC	<LOQ	<LOQ	0.01 (<LOQ;0.02)

(*continued on next page*)

Table 2 (*continued*)

Parent compound (CAS nr)	Biomarker	Analytical Method	Unit	LOD/ LOQ	Sample size	% above LOD/ LOQ	GM (95% CI)	P50	P90	P95 (95%CI)
Tris(1.3-dichloro-2-propyl) phosphate13674-87-8	Bis(1,3-dichloro-2-propyl) phosphate(BDClPP)			0.01 µg/L LOQ = 0.05 µg/L	413	80.4	0.29 (0.25;0.33)	0.33	1.52	2.93 (1.77;4.03)
Tri-n-butyl phosphate126-73-8	Di-n-butyl phosphate (DNBP)			LOQ = 0.15 µg/L	413	17.1	NC	<LOQ	0.22	0.35 (0.23;0.48)
Per- and polyfluoroalkyl substances in serum										
335-67-1	Perfluorooctanoic acid (PFOA)	UPLC-MS/MS (Supplemental Materials S1)	µg/L	LOQ = 0.2 µg/L	410	100	1.03 (1.00;1.07)	1.00	1.60	1.80 (1.70;2.00)
1763-23-1	Perfluorooctane sulfonate (PFOS)			LOQ = 0.2 µg/L	410	100	2.16 (2.02;2.30)	2.10	4.95	7.30 (6.10;8.00)
355-46-4	perfluoro-1-hexanesulfonate (PFHxS)			LOQ = 0.2 µg/L	410	96.6	0.48 (0.46;0.51)	0.48	0.97	1.30 (1.20;1.60)
375-95-1	Perfluorononanoic acid (PFNA)			LOQ = 0.2 µg/L	410	82.2	0.31 (0.30;0.32)	0.31	0.59	0.70 (0.63;0.86)
375-73-5	perfluoro-1-butanesulfonate (PFBS)			LOQ = 0.2 µg/L	410	0	NC	<LOQ	<LOQ	<LOQ
2706-90-3	perfluoro-n-pentanoic acid (PFPeA)			LOQ = 0.2 µg/L	410	0	NC	<LOQ	<LOQ	<LOQ
307-24-4	perfluoro-n-hexanoic acid (PFHxA)			LOQ = 0.2 µg/L	410	5.37	NC	<LOQ	<LOQ	<LOQ
375-85-9	Perfluoroheptanoic acid (PFHpA)			LOQ = 0.2 µg/L	410	1.22	NC	<LOQ	<LOQ	<LOQ
335-76-2	Perfluorodecanoic acid (PFDA)			LOQ = 0.2 µg/L	410	42.2	NC	<LOQ	0.38	0.49 (0.42;0.63)
2058-94-8	perfluoro-n-undecanoic acid (PFU(n)DA)			LOQ = 0.2 µg/L	410	7.56	NC	<LOQ	<LOQ	<LOQ
335-76-2	Perfluorodeconaoic acid (PFDoODA)			LOQ = 0.2 µg/L	410	1.22	NC	<LOQ	<LOQ	<LOQ
60270-55-5	perfluoro-heptanesulfonate (PFHpS)			LOQ = 0.2 µg/L	410	2.44	NC	<LOQ	<LOQ	<LOQ

GM: geometric mean, LOD: limit of detection, LOQ: limit of quantification, NC: geometric mean was not calculated because less than 60% of the samples could be quantified. UPLC-MS/MS: ultra-high-performance liquid chromatography with tandem mass spectrometry, HPLC-UV: high-performance liquid chromatography with ultra-violet spectroscopy, HR-ICP-MS: High Resolution Inductively Coupled Plasma Mass Spectrometry, HPLC-TQMS: high-performance liquid chromatography/triple quadrupole mass spectrometry, GC-MS/MS: gas chromatography with high-resolution mass spectrometry, LC-MS/MS: liquid chromatography with high-resolution mass spectrometry.

and neighborhood urbanicity.

3. Results and discussion

3.1. Study population

Forty-seven schools were contacted and 43% (n = 20) of them responded positively. Overall, 34% of the invited teenagers agreed to participate. The sociodemographic, lifestyle and residential characteristics of the study participants are summarised in Table 1 and compared with the characteristics of the general Flemish population for this age group. Our study population consisted of 428 teenagers. Its composition reflected the general population of the same age group with slightly more girls compared to boys, but distribution between the sexes is close to being equal. There were relatively more inhabitants of rural areas. The household educational attainment was higher in our study population as seen in previous FLEHS studies (Morrens et al., 2017) but representation improved over time thanks to measures that lowered thresholds to participate. The distribution over school types of the participants accorded well with that of Flanders in general. A minority of 8.1% of teenagers and/or a parent was born in another EU country, 11.1% was born outside the EU. Only 2.3% were daily smokers, which is a decrease compared to previous FLEHS studies and in line with the general Flemish trends. About one-third of the participants reported being exposed to environmental tobacco smoke. Fewer participants consumed alcohol weekly than in the general population and compared to previous FLEHS studies. The distribution of BMI in categories was following data from the general population with a mean BMI of 21.0 kg/m^2 and a standard deviation (SD) of 3.7 kg/m^2. Because recruitment was carried out in collaboration with the schools, no samples were

Table 3
Environmental chemicals, their respective biomarkers and exposure health-based guidance values applied in the FLEHS IV study.

Environmental chemical	Biomarker	Type of Health based guidance value (HB-GV)	Corresponding toxic endpoint	Unit	Health based guidance value	% participants exceeding HB-GV
Metals and trace elements						
Arsenic	U-TRA	BE	Hyperpigmentation and vascular effects	µg/L	6.4	25
Cadmium	U- Cd	HBM-GV	Kidney functioning	µg/g Cr	0.2	39.5
Thallium	U- Tl	HBM I	Fatigue, Sleeplessness	µg/L	5	0
Lead	B-Pb	EFSA	Developmental neurotoxicity	µg/L	12	12
		ECHA	Other effects		45	0
Pesticides						
Pyrethroïds	U-PBA	BE	Neurotoxicity	µg/L	1.7	22
					87	0
Chlorpyrifos	U-TCPγ	BGV	Cholinesterase inhibition	µg/L	520	0
2,4-dichloro Phenoxyaceticacid	U-2,4D	BE	Kidney toxicity	µg/L	10500	0
Polychlorinated biphenyls and halogenated pesticides						
PCBs	S- PCB	HBM I	Liver toxicity	ng/L	3500	0
		HBM II			7000	0
HCB	S-HCB	BE	Liver toxicity	ng/g vet	25	0.5
DDT	S-DDT + DDE + DDD	BE	Liver toxicity	ng/g vet	5000	0
Bisphenols and phthalates						
Bisphenol A	U-BPA	HBM-GV	Kidney weight	µg/L	230	0
DEP	U-MEP		Growth retardation	µg/L	18000	0
DnBP	U-MnBP	HBM-GV	Male reprotox	µg/L	190	0.5
DiBP	U-MiBP	HBM-GV		µg/L	230	1.9
BBzP	U-MBzP	HBM-GV		µg/L	3000	0
DEHP	U-5OH-MEHP + 5oxo-MEHP	HBM-GV		µg/L	500	0
Per- en polyfluorinated substances						
PFOA + PFNA	S-PFOA + S-PFNA	EFSA	Vaccination response	ng/mL	2	4
PFOS + PFHxS	S-PFOS + S-PFHxS	EFSA	Vaccination response	ng/mL	4.9	10

Health-based guidance values: HB-GV derived by HBM4EU for U-Cd (Lamkarkach et al., 2021), U-BPA (Apel et al., 2020b), U-MnBP, U-MiBP, U-MBzP, U-5OH-MEHP+5oxo-M (Lange et al., 2021b), BE value for U-TRA (Hays et al., 2010), BE for 3-PBA (Aylward et al., 2018), U-2,4D (Aylward and Hays, 2015), BE for S-HCB lower value corresponds to an external limit value of 0.05 µg/kg.day set by Health Canada (Aylward et al., 2010), BE for S-DDT + DDE corresponds to an external limit value of 0.5 µg/kg-day (Kirman et al., 2011), BGV for TCPγ (Arnold et al., 2015), BE for U-MEP (Aylward et al., 2009), B-Pb limit values derived from EFSA opinion 2013 ("Scientific Opinion on Lead in Food | European Food Safety Authority,")and RAC 2019 (European Chemical Agency (ECHA), 2018), limit values for S-PFAS derived from EFSA (Schrenk et al., 2020), HBM I for U-Tl from German HBM (Schulz et al., 2011), HBM I and HBM II for sum marker PCBs (PCB138, PCB153, PCB180) from German HBM (Apel et al., 2017).

collected during the summer season.

3.1.1. *Biomonitoring results*

For each chemical, Table 2 presents summary statistics from the FLEHS IV HBM program for a relevant biomarker (parent chemical,

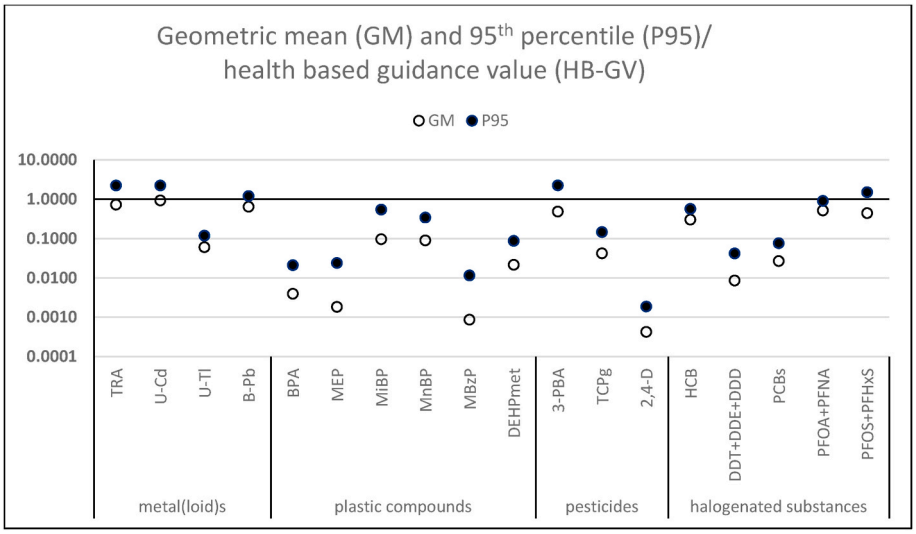


Fig. 1. Chemical specific risk coefficients based on comparisons of internal exposures with corresponding health based guidance values.

metabolite or sum of metabolites). Urinary markers were normalized to SG to adjust for urinary dilution. Urinary biomarker data normalized to Cr are presented in Supplemental Materials S2 and the volume based biomarker data (blood and urine) in Supplemental Materials S3.

HBM-GVs are listed in Table 3 and the proportion of the study participants that exceeded these guidance values is indicated. Sometimes, different HB-GVs for the same biomarker are listed reflecting the differences in opinions of risk assessors and risk assessment committees in expressing exposure-related health concerns. The HB-GVs that are considered refer only to non-cancer endpoints. Fig. 1 shows the risk coefficient (RQ) as the GM and P95 divided by the most sensitive HB-GV for the chemical-specific biomarker.

Fig. 2 displays changes in biomarker levels over time with the GM biomarker concentrations expressed as percentages of the concentrations at the first measuring point. Several biomarkers have been measured in the same age group in previous FLEHS studies, starting from 2002.

3.2. Metals and trace elements

Cd, Pb, As and Tl were included as metals. B-Pb and B-Cd were measured in all previous FLEHS cycles, toxicologically relevant arsenic (TRA) was included in FLEHS II and FLEHS III, U-Tl in FLEHS II. Levels of B-Pb decreased compared to the previous FLEHS studies (Fig. 2A). Success may relate to continued efforts to implement measures such as changing waterpipes in old houses. Mean biomarker levels of B-Cd and TRA remained stable, while U-Tl increased slightly. In some industrial and historically contaminated Flemish areas, elevated concentrations of cadmium and lead in soil still exist. Thallium is an emerging metal used increasingly for electronic and wireless applications and may be emitted near smelters, power plants and cement factories. It may enter different environmental compartments including the food chain (Aprea et al., 2020). Levels of naturally occurring As in groundwater raise concern for home grown food consumption and the use of well water as drinking or showering water (Monteiro De Oliveira et al., 2021).

Between 12 and 39.5% of the participants exceeded the HB-GVs for exposure to TRA, U-Cd, B-Pb (Table 3) and adverse health outcomes cannot be excluded. The GM of U-Cd, B-Pb and U-TRA are close to the HB-GVs (Fig. 1). Cd and As are classified by IARC as known human carcinogens (Group I) and Pb as a possible human carcinogen (Group 2B)(International Agency for Research on Cancer, 2019). No exceedance was observed for U-Tl, there are few population studies, but health effects at low Tl levels are reported, especially there is a concern for neurotoxicity (Campanella et al., 2019).

B-Tl and B-Cd were higher in teenagers from lower educated households but significance disappeared after adjustment for smoking. The positive association of TRA with the family's educational attainment was borderline significant ($p < 0.10$). A similar association with high SES has been described in the HBM program of the US-Centers for Disease Control and Prevention (US-CDC) and was mediated by more fish and shellfish consumption (Tyrrell et al., 2013). B-Pb and blood manganese(B-Mn) were borderline ($p < 0.10$) associated with residence area, with B-Pb higher in residents from rural areas and B-Mn higher in residents from towns and suburbs (Fig. 4). Underlying causes are not clear but may relate to living in older houses with leaded waterpipes.

U-Cd, U-TRA and B-Cu were higher in girls, B-Pb and B-TI were higher in boys after adjustment for household educational attainment, urbanization characteristics, sampling season, age of the participants.

FLEHS IV GM of B-Cd and B-Pb are higher than reported by US-CDC and Health Canada for sampling in the same period (>2015) in a comparable age group (12–19 years old), while the GM TRA levels of FLEHS IV are similar (US-CDC) (Health Canada, 2019). Comparison with HBM programs in Europe (VITO, 2021), showed that GM of TRA is lower than reported for 7 year-old children from Northeast Italy (Bocca et al., 2020), P50 B-Cd and B-Pb of FLEHS IV are lower than those measured in adults and in children sampled in the Czech HBM program of 2015–2016 (Černá et al., 2017), P50 B-Pb of FLEHS IV and GerES V teenagers (14–17 years) were similar but P50 B-Cd was higher in FLEHS IV teenagers (Vogel et al., 2021) (Supplemental materials S4).

3.3. Combustion-related compounds

Benzene and PAHs are considered relevant for spatial planning as they relate to traffic exhaust, industrial emissions and heating of buildings. PAHs are also a relevant group of compounds in the indoor environment, as they are produced during cooking and heating (IARC Working Group on the Evaluation of Carcinogenic Risks, 2010). We have biomonitoring data from previous FLEHS cycles for 1-OH PYR as pyrene metabolite and for t,t'-MA as benzene metabolite in urine. We included additional metabolites for some smaller volatile PAHs in the current study: NAPH, PHE and FLU. 2-OH NAPH is the most abundant biomarker of the low molecular weight PAHs (Table 2).

GM levels of 1-OH PYR have decreased for the first time in the successive FLEHS studies, while levels of t,t'-MA increased (Fig. 2B). This coincides with a decreasing trend in air emissions of PAHs in Flanders that was reported in 2014 for the first time since 2000, while the benzene concentrations in the air decreased until 2014, but showed a slight increase thereafter (Vlaamse Milieumaatschappij, 2017).

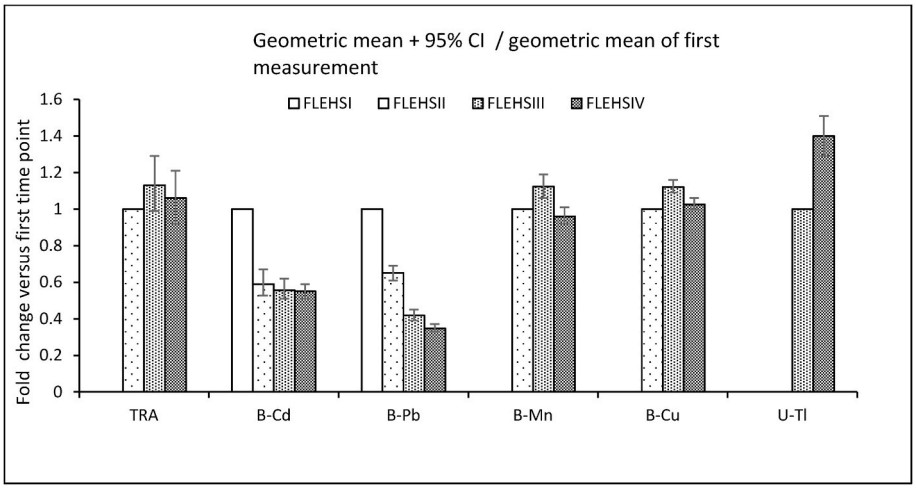


Fig. 2A. Changes over time of urinary biomarkers (U) of trace elements adjusted for sex, smoking behavior, age, creatinine; blood biomarkers (B) adjusted for sex, age and smoking behavior.

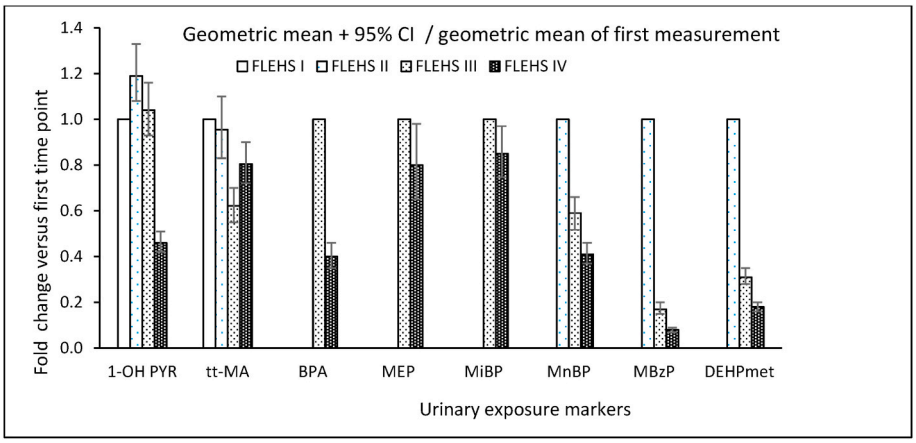


Fig. 2B. Changes over time of urinary biomarkers for combustion products and plastic compounds adjusted for sex, smoking behavior, age, body mass index (BMI) and creatinine.

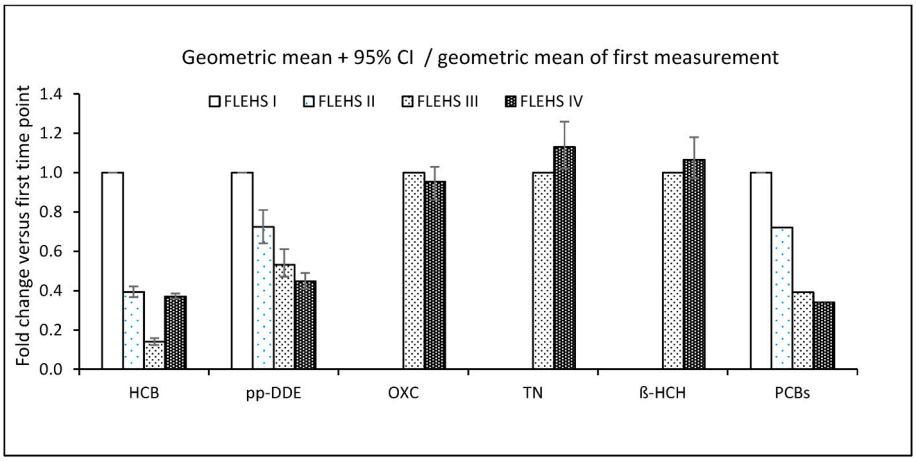


Fig. 2C. Changes over time of serum biomarkers of halogenated substances adjusted for sex, smoking behavior, age, BMI and serum lipids.

No HB-GVs are available, but both substances are classified as known human carcinogens by IARC (Group I)(International Agency for Research on Cancer, 2019). Based on annual air concentrations, extra cancer risks were estimated for benzene between 1/156 000 and 1/587 000 and for benzo-a-pyrene between 1/50 000 and 1/210 000 depending on the place of residence and if levels would remain the same in the future (Vlaamse Milieumaatschappij, 2020). FLEHS IV study results indicate that adverse effects on the adolescent endocrine and immune system cannot be excluded(Verheyen et al., 2021).

Several of the PAH metabolites (1-OH PYR, sum of 2-OH FLU and 3-OH FLU, 2-OH PHE) and also t,t'-MA were elevated in teenagers from lower-educated households (Fig. 3). We also observed higher levels of 1-

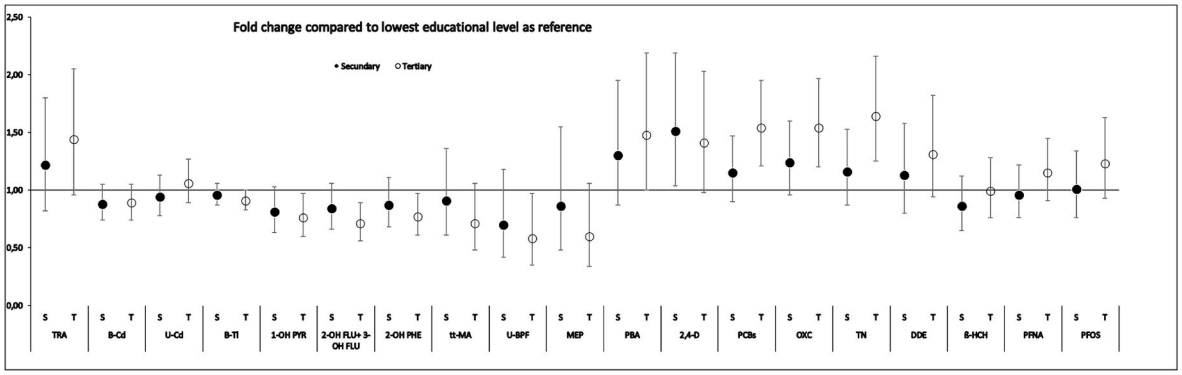


Fig. 3. Biomarkers that show significant ($p < 0.10$) associations with highest educational level attainment of the household: primary (no educational attainment, primary school, lower secondary school), secondary (higher secondary school) and tertiary (higher education attainment). These levels correspond to the codes 0–2, 3–4 and 5–8 of the International Standard Classification of Education (ISCED). The primary educational level is used as reference category. Fold change and 95% CI are presented. $P < 0.05$ for U-Cd, B-Tl, 2-OH NAPH, sum of 2-OH FLU and 3-OH FLU, 2-OH PHE, tt'-MA, MEP, BPF, PCBs, OXC, TN, HCB, PFNA and PFOS and borderline significant ($p < 0.10$) for TRA, 1-OH PYR, the pesticide markers 3-PBA, 2,4-D, DDE, β-HCH.

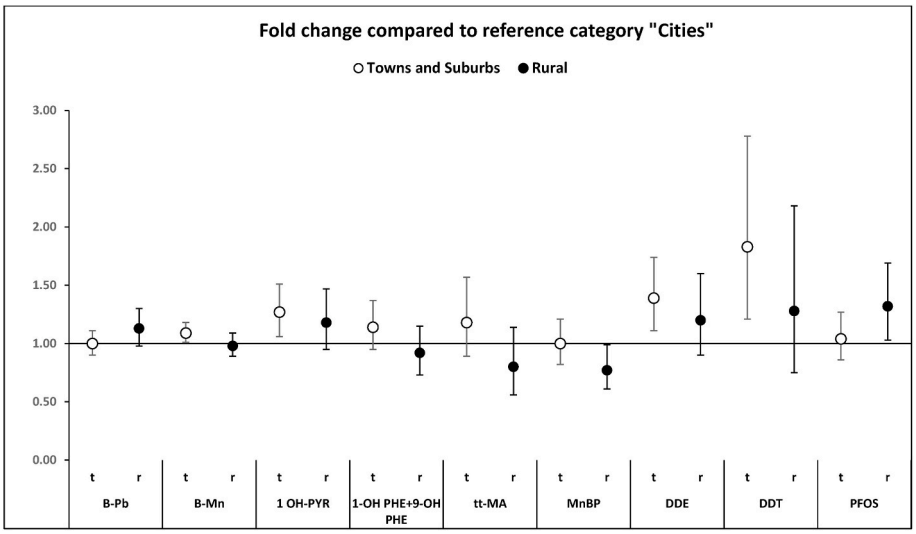


Fig. 4. Biomarkers levels that are associated ($p < 0.10$) with the neighborhood urbanicity of the participants. The neighborhoods are categorized according to the EUROSTAT definitions and "cities "are used as the reference category. Fold change and 95%CI are presented.

OH PYR, the sum of 1 and 9-OH PHE and t,t'-MA in residents from towns and suburbs. Fig. 4 displays the fold change of these biomarkers (GM and 95% CI) with cities as the reference category. Significance ($p < 0.05$) remained after adjustment for smoking. Exposure to environmental tobacco smoke (ETS) at home, living close to industry and heavy traffic have been suggested as exposure determinants for PAHs (Iamiceli et al., 2020; Sochacka-Tatara et al., 2018). Levels of 2-OH NAPH were higher in girls than in boys.

We compared with large representative international studies from similar age groups and sampling periods (Supplemental material S2). The urinary GM/P50 levels in this study were below the ones reported by the HBM programs of Health Canada with sampling in 12–19 years old teenagers in 2014–2015 (Health Canada, 2017) and were similar or lower than reported by GerES V for 14–17 years old teenagers with sampling in 2014–2017 (Murawski et al., 2020).

3.4. Plastic compounds

Bisphenols have been used to produce polycarbonate plastics and epoxyresins and are present in various consumer products including toys, food packaging materials, food cans (Vandenberg et al., 2007). Phthalates are added to soften plastics and are also used in a lot of consumer products. People may be exposed by dermal contact, by food and by inhalation as these compounds settle on house dust (Luis et al., 2021). BPA, Diethyl phthalate (DEP), Dibutyl phthalate (DnBP), Diisobutyl phthalate (DiBP), Benzyl butyl phthalate (BBzP) and Bis (2-ethylhexyl) phthalate (DEHP) are regulated under REACH at the EU level (ECHA, 2018). As reported earlier (Gys et al., 2021), three of the six measured bisphenols could be quantified in more than 60% of teenagers. Levels of BPA were higher than those of PBF and BPS. Only BPA was measured in FLEHS III, the GM in the FLEHS III study was almost double compared to the FLEHS IV study (Fig. 2B). No significant health risk for BPA is expected as the recently derived HB-GVs are not exceeded (Apel et al., 2020a), no HB-GVs are published for the other bisphenols. Only BPF was higher in teenagers from lower educated households but not the other bisphenols. Also a German study reported higher BPA levels in children from families with lower SES status which was a composite variable based on parent's income, education, and profession (Tschersich et al., 2021).

The levels of BPA are in the same range as those found in other studies from a comparable time frame. The P95 BPA and BPF levels (volume based) are somewhat lower than the HBM reference values for children (6–17 years old) measured in the French Esteban study (2014–2016) (Fillol et al., 2021), while BPS levels were roughly ten times lower. P50 BPA levels (volume based) found in German teenagers (14–17 years old) (GerESV 2014–2017) are higher (Tschersich et al., 2021).

MEP showed the highest GM concentration followed by MiBP, MnBP and cx-MEPP. Significant decreases in GM levels of regulated phthalates (DEHP, DiBP, DnBP and DBzP) were observed compared to the levels measured in the previous FLEHS III study (Fig. 2B). Many consumer products are labelled and consumers nowadays often have the choice to buy BPA-free or phthalate-free products. The recently derived HB-GVs of DnBP and DiBP were exceeded in respectively 0.5 and 1.9% of the participants (Table 3), while no exceedances were measured for DEHP metabolites, demonstrating the efficacy of regulation(Lange et al., 2021a).

The results confirmed earlier observations that MEP, which is mainly used in personal care products, showed higher levels in teenagers from lower educated households (Fig. 3) (Den Hond et al., 2015). MnBP levels were lower in residents from rural areas compared to cities. SES status and degree of urbanization should be considered as surrogate markers for specific behaviors as discussed extensively before (Bastiaensen et al., 2021a). After adjustment for household educational attainment, urbanization characteristics, sampling season, age of the participants BPA, MEP, MnBP and MEHP levels were higher in girls than in boys. This may relate to differences in exposures or metabolism. The demand for plastics and plasticizers remains and a comprehensive study revealed that metabolites of substitute plasticizers such as di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH), di-(2-ethylhexyl) terephthalate (DEHTP) and di-(2-ethylhexyl) adipate (DEHA) could be quantified in urine samples of Flemish teenagers (Bastiaensen et al., 2021a). The toxicological database for these substitutes is not yet complete and their impact on human health is not fully understood.

We reported earlier (Bastiaensen et al., 2021a) that concentrations of phthalate metabolites measured in this study were generally consistent with studies of adolescents from Canada (Health Canada, 2019) and Germany (Schwedler et al., 2020). Our P95 volume based levels for MiBP, MEP, MBzP, MnBP and the DEHP metabolites were below the French reference values for 6–17 years old children (Fillol et al., 2021) (Supplement S4).

3.5. Pesticides

Biomarkers for pyrethroids, chlorpyrifos, the phenoxyherbicide 2,4-D and glyphosate were measured for the first time in spot urine samples

of a representative sample of Flemish teenagers. No time trends for internal exposures in the Flemish region are yet available. These short-lived pesticides are used professionally in agricultural areas but also privately in homes and gardens. Residues present in commercially bought food and locally grown food may also expose the general population. According to FAOSTAT, pesticide use per area of cropland in Belgium (6.96 kg/ha) is about four times above the European average (1.66 kg/ha) (FAO, 2019).

Pyrethroids are synthetic pesticides that are increasingly used as insecticides in public and private places indoors and outdoors and replace some of the banned halogenated pesticides. The potential for human exposure is high, both from the intake of residues in food items and by dermal and inhalation exposures via direct contact and from dust (Morgan, 2012). They are regulated at the EU level in food and as plant protection products (Andersen H.R., 2021). We measured 3-phenoxybenzoic acid (3-PBA) which is a common metabolite of several widely used pyrethroids (Cypermethrin, Deltamethrin, Permethrin, lambda-Cyhalothrin, D-Phenothrin and D-Phenothrin Fluvalinate-tau). 3-PBA could be quantified in nearly all urine samples.

Chlorpyrifos was one of the most used organophosphate insecticides, but in 2017 its use was limited to certain applications in Flanders. At EU level it is no longer approved since 2019 (Andersen H.R., 2021). The chlorpyrifos metabolite, TCPγ, could still be detected in all samples from FLEHS IV teenagers. It is anticipated that levels will decrease due to the recent regulations.

GLY is worldwide the most used herbicide. The use of GLY and its degradation product AMPA by non-professionals has been restricted in Flanders since 2018, but it is still primarily used by farmers. GLY and the AMPA metabolite could be measured in respectively 41.45 and 55.9% of the FLEHS IV teenagers (LOQ 0.1 μg/L). Flemish adolescents with GLY below LOQ had on average more diluted urine samples (SG of 1.024703) versus samples with GLY above LOQ (SG of 1.020276). This underestimates the fraction of exposed participants. The average dilution factor, calculated by the formula (1.024-1)/(SG-1), was 1.22 fold higher for samples below LOQ than for samples above LOQ. If the same dilution factor would be applied, 45 samples out of 172 with values above LOQ would be no longer quantifiable.

Although results of spot urine samples of short-lived biomarkers should be interpreted with caution (Bastiaensen et al., 2021c), 22% of the study participants exceeded the most sensitive HB-GV for 3-PBA (Table 3). The most sensitive HB-GV assumes that all parent compounds have the same toxic potency as the known most toxic compound. Neurotoxic and endocrine effects are health concerns associated with pyrethroids and chlorpyrifos (Koureas et al., 2012; Saillenfait et al., 2015), while 2,4-D is classified as possible human carcinogen (Group 2B) (International Agency for Research on Cancer, 2019). IARC has classified GLY as probably carcinogenic (Group 2A) (International Agency for Research on Cancer, 2019), while the EU considers its aquatic toxicity as the basis for regulation (Kalofiri et al., 2021).

Levels of 2,4-D and 3-PBA were higher in the teenagers from higher educated households (Fig. 3). Higher levels of pyrethroid metabolites in relation with higher household educational attainment were also observed in an Italian study (Bravo et al., 2019) but not in other studies (Fernández et al., 2020a; Norén et al., 2020; Rodzaj et al., 2021).

The P50 of the pyrethroid biomarkers 3-PBA of FLEHS IV teenagers is higher than reported for adolescents from Sweden (Norén et al., 2020), 12-19 year-old teenagers from Canada (Health Canada, 2019), Polish urban-dwelling young adults (Rodzaj et al., 2021), children in Trieste (Bravo et al., 2019) but lower than those of children in the Spanish Valencian region (Fernández et al., 2020) and comparable to children from the Belgian Walloon region (Pirard et al., 2020). The P50 chlorpyrifos metabolite TCPγ, is higher in FLEHS IV teenagers than reported in Italian, Spanish and Swedish studies (Supplemental materials S4). The P95 of 2,4-D is higher in Flemish teenagers than in Swedish adolescents (Norén et al., 2020), but lower than in Spanish children (Fernández et al., 2020). The detection frequency of GLY and AMPA with quantification limit of 0.1 μg/L, was comparable to German children with GLY and AMPA quantified in respectively 52% and 46% of (GerESV 2014–2017) samples (Lemke et al., 2021) using the same analytical method and LOQ. A recent study of Slovenia reported, depending on the sampling season, between 22 and 27% detects for GLY and between 50 and 56% for AMPA in children and teenagers between 7 and 15 years old, LOQ was also 0.1 μg/L (Lemke et al., 2021; Stajnko et al., 2020).

Metabolites of DDT, lindane (β-HCH), the chlordane related compounds and HCB were detected in the majority of serum samples of FLEHS IV teenagers. Chlorinated pesticides are legacy chemicals as their production and use is eliminated or restricted under the Stockholm convention; however these chemicals are still present in the environment and the food chain. Food is the primary exposure source for the general population (Keswani et al., 2021). OXC, TN and HCHs were measured in teenagers of FLEHS III with sampling in 2013. DDT, DDE and HCB were measured in all the previous FLEHS studies with sampling in 2003–2004 (FLEHS I), 2007–2008 (FLEHS II), 2013 (FLEHS III). Only levels of DDE are gradually decreasing over the different FLEHS studies, but decreases in concentrations could not be observed for the other halogenated pesticides (Fig. 2 C).

HB-GVs relate to the endocrine disrupting properties associated with these compounds (Gheidarloo et al., 2020). GM and P95 did not exceed HB-GVs. Only a few individuals exceeded the HB-GV of HCB (Table 3). However, significant exposure-effect associations have been observed with HCB and DDT metabolites in earlier FLEHS studies (Croes et al., 2015). The carcinogenic properties of these compounds remain of concern as IARC classified DDT as probably carcinogenic (Group 2A), HCB, chlordane and HCH isomers as possibly carcinogenic (Group 2B) (International Agency for Research on Cancer, 2019).

The household educational attainment explained a part of the variability of OXC, TN, HCB ($p < 0.05$) and of DDE, β-HCH ($p < 0.10$) as shown by multiple regression models adjusted for age, sex, sampling season and blood lipids. Significance for DDE and β-HCH disappeared after correction for BMI and being breastfed. Mean biomarker levels of OXC, TN, HCB and DDE were highest at the highest educational attainment (Fig. 3). Previous studies have observed similar associations for the halogenated compounds (Bandow et al., 2020; Morrens et al., 2012) and these were associated with higher intake of egg products, meat and fish (Arrebola et al., 2018). DDE and DDT biomarker levels were significantly higher in teenagers from towns and suburban areas than from cities (Fig. 4). Higher consumption of locally produced eggs and food have been associated with a higher burden of halogenated substances in previous FLEHS cycles (Colles et al., 2021; Den Hond et al., 2009).

Compared to girls, boys had higher biomarker levels of HCB, DDE, β-HCH, OXC and TN after adjustment for household educational attainment, urbanization characteristics, sampling season, age of the participants.

Recent HBM data from the German children and adolescent study (GerESV 2014–2017) revealed similar GM of HCB, β-HCH was twice as high in Germany, while DDE was 56% higher in FLEHS IV participants (Bandow et al., 2020).

3.6. PCBs and flame retardants

Marker PCBs were quantifiable in serum of all teenagers of FLEHS IV and were measured in the same age group in the three previous FLEHS studies. GM PCB levels decreased in FLEHS IV compared to the previous FLEHS III study (Fig. 2C). None of the BFR congeners could be quantified in more than 60% of the participants (only 4 mL serum samples were available for the analysis of the halogenated chemicals). We noticed that the detection frequency of PBDE153 decreased from 61,4% in FLEHS II to 38% in FLEHS III to 21.3% in FLEHS IV applying the same LOQ. HB-GVs of PCBs, based on liver toxicity, were not exceeded in the study participants (Table 3). IARC classified PCBs in group I (International Agency for Research on Cancer, 2019).

PCBs and BFRs are another class of legacy chemicals that are regulated under the Stockholm convention. PCBs have cooling properties and were produced for specific applications, while PBDEs were used as flame retardants. These chemicals are banned, but remain circulating in the environment and the general population is exposed mainly to food (Lebelo et al., 2021).

As observed for some other biomarkers of chlorinated chemicals, teenagers of higher educated households had higher PCB burdens (Fig. 3). Levels of PCBs were higher in boys than in girls after adjustment for household educational attainment, urbanization characteristics, sampling season, age of the participants.

Compared with German children and teenagers (GerES 2014–2017) (Bandow et al., 2020), PCBs in Flemish teenagers were somewhat lower if GM are compared (Supplemental Materials S4).

BFRs are being replaced by alternatives such as OPFRs. These chemicals are used as flame retardants but also as plasticizers in a wide variety of consumer products such as furniture, textiles, decoration and building materials, paints, floor polish, resins and polyvinyl chloride plastics (Chokwe et al., 2020). Six biomarkers of five OPFRs were found in practically all participants. The GM and P95 were highest for 2-ethylhexyl phenyl phosphate (EHPHP), a biomarker for 2-ethylhexyl diphenyl phosphate. No HB-GVs were available, as information on the toxicology of these compounds is still limited. Some of these chemicals are suspected developmental and carcinogenic toxicants (Alzualde et al., 2018; Wei et al., 2020). No significant associations with household educational attainment or urbanicity have been observed in this reference population. A more extended study that included 582 participants has identified higher levels of BBOEHEP and BDCIPP in teenagers from higher educated households (Bastiaensen et al., 2021b).

3.7. Per- and polyfluoroalkyl substances

PFOS and PFOA were quantified in all serum samples, demonstrating ubiquitous exposure. PFOS showed the highest concentrations followed by PFOA, while levels of PFNA and PFHxS were lower. Perfluoro-n-decanoic acid (PFDA) could be quantified in 42.2% of the participants, while the concentrations of the other PFAS were mostly below the LOQ of 0.2 μg/L. The PFAS are a large group of compounds with interesting properties: water, dirt and oil repellent. They are applied in a diversity of consumer products, but food may also be an exposure source for humans. Their persistency in the environment, accumulation and long half-life-in humans are a growing concern (Fromme et al., 2009). Our HBM-data may be compared with internal concentrations that correspond with the recently by EFSA proposed intake limits based on a reduced vaccination response as critical effect (Schrenk et al., 2020). Respectively 10 and 4% of our participants exceeded these values for the sum of PFOS and PFHxS (4.9 ng/mL), and for the sum of PFOA and PFNA (2 ng/mL) (Table 3). Various health effects including developmental effects, liver toxicity, immune effects have been associated with these persistent compounds and their endocrine properties (Rappazzo et al., 2017). IARC classified PFOA in group 2B (International Agency for Research on Cancer, 2019). Regulation in the EU has restricted the production and use of PFOS and PFOA in a variety of applications (ECHA, 2021), while recent risk assessment made by EFSA proposed to regulate the sum of four substances: PFOA, PFOS, PFHXS, PFNA (Schrenk et al., 2020). Although decreasing levels for PFOS and PFOA have been described earlier in Flemish newborns (Colles et al., 2020), our new study demonstrates that concerns for adverse health effects cannot be excluded. Moreover, we have measured only a very small fraction of the fluorinated compounds that enter the market and many replacements of the restricted compounds have not been measured in human matrices yet (Kaiser et al., 2021).

Teenagers from higher educated households showed higher levels of PFOS and PFNA (Fig. 3). This confirms results of higher PFAS levels associated with higher socio-economic position described in German children and adolescents (Duffek et al., 2020), in children and pregnant mothers from European urban birth cohorts (Montazeri et al., 2019) and in relation with higher income as found in a meta-analysis including five HBM studies (Buekers et al., 2018). Differences in breastfeeding and dietary patterns have been suggested as underlying reasons (Buekers et al., 2018; Duffek et al., 2020). We observed higher levels of PFOS in residents from rural areas compared to those from cities (Fig. 4). Earlier studies in Flanders have shown that higher consumption of locally grown foods occurs more in rural areas and is associated with higher internal exposure levels of several persistent compounds such as PFOS and PFOA (Colles et al., 2020). Levels of PFOA and PFHXs were higher in boys than in girls.

The GM serum levels of PFOS, PFOA, PFHxS and PFNA showed similar values compared to recently published values from French and Canadian children (Fillol et al., 2021; Health Canada, 2019) and from German teenagers (Duffek et al., 2020) (Supplemental Materials S4).

3.8. Strengths and weaknesses

Our aim was to recruit a population sample of 14- and 15-year old teenagers that was representative for geographical location and sex and included participants from different socio-economic strata and residential areas with varying degrees of urbanization. We compared our population sample with available population characteristics of Flemish teenagers. We found some overrepresentation of teenagers from rural areas but a good match for most other characteristics including SES. Accordingly, no extra weighing factors were introduced as we consider our population exposure estimates to represent 14–15 year' old Flemish teenagers. We could not sample in the summer months as schools (our primary sampling units) were closed. Dietary habits and life style may change with season. Season of sampling was a significant covariate for several biomarkers. Not sampling equally in all seasons may have somewhat biased representativity of the biomarkers. To assess impact of educational attainment of the household and degree of urbanization of the residence area, the statistical models were adjusted for season of sampling.

We have compared our exposure distributions with other general population studies that sampled teenagers of approximately the same age as exposures may differ according to age (Supplemental Materials S4). Also, the sampling years should be comparable as chemical production, use patterns and regulations change over time. Only a few countries carried out HBM studies of a representative sample of the general population after 2015 and most studies reported HBM data from a more extended age group. Interpretations are hampered by differences in study and sampling design, laboratory analysis, reported LOQs and data handling. Further harmonization of study design and assessment of the comparability of biomarker analysis such as currently ongoing in Human Biomonitoring for Europe (HBM4EU) will benefit comparability of HBM data in Europe (Ganzleben et al., 2017).

Improved analytical techniques allowed quantifying a wide diversity of chemical compounds in the same individual. The presence of a chemical itself in serum or urine does not imply adverse health risks. Comparison of the biomarker data with corresponding HB-GVs was possible for only 18 of the 80 biomarkers. HB-GVs change regularly as more information becomes available; different organizations may come to different conclusions and handle different HB-GVs. HB-GVs are available for non-cancer endpoints. Some of the chemicals are classified by IARC as known (PAHs, benzene, Cd, As, PCBs), probable (GLY, DDT) or possible (Pb, 2,4-D, HCB, HCH, chlordanes, PFOA) human carcinogens and levels should be as low as reasonably achievable to avoid extra cancer risks.

Enhanced risks also assume chronic exposure at the same level throughout life.

Certainly for short-lived chemicals, such as pyrethroids and phthalates, the results of spot urine measurements may vary within an individual even within a day. More frequent sampling per individual is needed to interpret exposure data at the individual level. To assess

exposure determinants of these short-lived chemicals large sample sizes are needed. With more than 400 individuals (except for As biomarkers) sampled within a narrow age range, sample size was sufficient to assess the impact of important covariates such as the urbanicity of the residence area and the household educational attainment.

The present manuscript describes exposures and risks for individual chemicals. However, we acknowledge that increasingly complex chemical mixtures are detected in particular samples which will need new methods and approaches to quantify their risks.

4. Conclusions

The study emphasizes the success of chemicals' regulation: all recently restricted phthalates (DEHP, DiBP, DnBP, DBzP) and BPA biomarkers were lower than in the previous study (FLEHS III). Internal exposure to cadmium, lead decreased. The sum of PCBs was reduced to one third in 15 years. However, this was not seen for the chlordanes, with even significant increases of TN. Substitutes of regulated chemicals (PFAS, flame retardants and plasticizers) popped up in many samples. Little is known about their toxicity, warranting further follow up of the exposure levels in the population and toxicity information to prevent future health risks.

Adverse health risks at the population level are still expected for some traditional pollutants such as cadmium, lead, arsenic, polycyclic aromatic hydrocarbons, benzene and perfluoroalkyl substances, emphasizing the need for further measures and control of sources. Health risks cannot be excluded as more than 5% of the study participants exceeded HB-GVs of TRA, urinary Cd (U-Cd), blood Pb (B-Pb), pyrethroids and PFAS. The GM of U-Cd was close to the guidance values. GM and P95 of HCB, DiBP, DnBP and P95 of TCPγ and urinary Tl (U-Tl) were within one order of magnitude of the HB-GVs. A few individuals exceeded the guidance values of HCB, DnBP and DiBP.

We have specifically looked into the relationship between urbanization and internal exposure as Flanders is densely populated and urbanization is expected to further increase in the future. Household educational attainment and residential urbanicity influenced biomarker levels in both directions. Markers of several PAH metabolites, MeP and BPF were higher in teenagers from lower educated households, while biomarkers of halogenated substances were often higher in teenagers from higher educated households. PFOS was higher in residents of rural areas, while residents from towns and suburbs showed the highest levels of benzene and PAH metabolites but also of DDE and DDT. Underlying causes may relate to differences in environment, lifestyle and food habits and need further research to reduce internal exposure efficiently.

The Information on exposure will support prioritization and evaluation of policies and will inform on personal choices that people can make themselves.

Ethics

The study protocol was approved in June 2017 by the Antwerp University Hospital Ethical committee (Belgian Registry Number B300201732753).

Declaration of competing interest

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

Acknowledgements

We thank the teenagers and their families who participated in FLEHS-IV. Without their effort, this study would not have been possible. We thank the field workers from the Provincial Institute of Hygiene and VITO for the sample and data collection. We acknowledge the valuable input to the field work committee of Karen Van Campenhout and Caroline Teughels from the Flemish Department of Environment & Spatial Development. This paper is based on research conducted within the framework of the Flemish Center of Expertise on Environment and Health (FLEHS 2016–2020). The Flemish Center of Expertise on Environment and Health is funded by the Flemish Government, Department of Environment & Spatial Development. The views expressed herein are those of the author(s) and are not necessarily endorsed by the Flemish government. Analysis of phthalates and per-and polyfluoroalkyl substances were co-funded from the EU Horizon 2020 Framework Project HBM4EU, Grant Agreement No 733032.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113972.

References

Alzualde, A., Behl, M., Sipes, N.S., Hsieh, J.H., Alday, A., Tice, R.R., Paules, R.S., Muriana, A., Quevedo, C., 2018. Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicol. Teratol. 70, 40–50. https://doi.org/10.1016/J.NTT.2018.10.002.

Andersen, H.R., 2021. Pesticides – HBM4EU – science and policy for a healthy future [WWW Document], URL. https://www.hbm4eu.eu/hbm4eu-substances/pesticides/. (Accessed 12 September 2021).

Andersen, H.R., Dalsager, L., Jensen, I.K., Timmermann, C.A.G., Olesen, T.S., Trecca, F., Nielsen, F., Schoeters, G., Kyhl, H.B., Grandjean, P., Bilenberg, N., Bleses, D., Jensen, T.K., 2021. Prenatal exposure to pyrethroid and organophosphate insecticides and language development at age 20–36 months among children in the Odense Child Cohort. Int. J. Hyg Environ. Health 235. https://doi.org/10.1016/j.ijheh.2021.113755.

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int. J. Hyg Environ. Health 210, 201–228. https://doi.org/10.1016/J.IJHEH.2007.01.024.

Apel, P., Angerer, J., Wilhelm, M., Kolossa-Gehring, M., 2017. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. Int. J. Hyg Environ. Health 220, 152–166. https://doi.org/10.1016/j.ijheh.2016.09.007.

Apel, P., Rousselle, C., Lange, R., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2020a. Human biomonitoring initiative (HBM4EU) - strategy to derive human biomonitoring guidance values (HBM-GVs) for health risk assessment. Int. J. Hyg Environ. Health 230. https://doi.org/10.1016/j.ijheh.2020.113622.

Apel, P., Rousselle, C., Lange, R., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2020b. Human biomonitoring initiative (HBM4EU) - strategy to derive human biomonitoring guidance values (HBM-GVs) for health risk assessment. Int. J. Hyg Environ. Health 230, 113622. https://doi.org/10.1016/j.ijheh.2020.113622.

Aprea, M.C., Nuvolone, D., Petri, D., Voller, F., Bertelloni, S., Aragona, I., 2020. Human biomonitoring to assess exposure to thallium following the contamination of drinking water. PLoS One 15. https://doi.org/10.1371/journal.pone.0241223.

Arnold, S.M., Morriss, A., Velovitch, J., Juberg, D., Burns, C.J., Bartels, M., Aggarwal, M., Poet, T., Hays, S., Price, P., 2015. Derivation of human biomonitoring guidance values for chlorpyrifos using a physiologically based pharmacokinetic and pharmacodynamic model of cholinesterase inhibition. Regul. Toxicol. Pharmacol. 71, 235–243. https://doi.org/10.1016/j.yrtph.2014.12.013.

Arrebola, J.P., Castaño, A., Esteban, M., Bartolomé, M., Pérez-Gómez, B., Ramos, J.J., 2018. Differential contribution of animal and vegetable food items on persistent organic pollutant serum concentrations in Spanish adults. Data from BIOAMBIENT. ES project. Sci. Total Environ. 634, 235–242. https://doi.org/10.1016/j.scitotenv.2018.03.283.

Aylward, L.L., Hays, S.M., 2015. Interpreting biomonitoring data for 2,4-dichlorophenoxyacetic acid: update to Biomonitoring Equivalents and population biomonitoring data. Regul. Toxicol. Pharmacol. 73, 765–769. https://doi.org/10.1016/j.yrtph.2015.11.001.

Aylward, L.L., Hays, S.M., Gagné, M., Krishnan, K., 2009. Derivation of Biomonitoring Equivalents for di-n-butyl phthalate (DBP), benzylbutyl phthalate (BzBP), and diethyl phthalate (DEP). Regul. Toxicol. Pharmacol. 55, 259–267. https://doi.org/10.1016/j.yrtph.2009.09.003.

Aylward, L.L., Hays, S.M., Gagné, M., Nong, A., Krishnan, K., 2010. Biomonitoring equivalents for hexachlorobenzene. Regul. Toxicol. Pharmacol. 58, 25–32. https://doi.org/10.1016/j.yrtph.2010.06.003.

Aylward, L.L., Irwin, K., St-Amand, A., Nong, A., Hays, S.M., 2018. Screening-level Biomonitoring Equivalents for tiered interpretation of urinary 3-phenoxybenzoic acid (3-PBA) in a risk assessment context. Regul. Toxicol. Pharmacol. 92, 29–38. https://doi.org/10.1016/j.yrtph.2017.11.002.

Bandow, N., Conrad, A., Kolossa-Gehring, M., Murawski, A., Sawal, G., 2020. Polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP) in blood plasma – results of the German environmental survey for children and adolescents 2014–2017 (GerES V). Int. J. Hyg Environ. Health 224. https://doi.org/10.1016/j.ijheh.2019.113426.

Barbanel, C.S., Winkelman, J.W., Fischer, G.A., King, A.J., 2002. Confirmation of the Department of Transportation criteria for a substituted urine specimen. J. Occup. Environ. Med. 44, 407–416. https://doi.org/10.1097/00043764-200205000-00009.

Bastiaensen, M., Gys, C., Colles, A., Malarvannan, G., Verheyen, V., Koppen, G., Govarts, E., Bruckers, L., Morrens, B., Francken, C., Den Hond, E., Schoeters, G., Covaci, A., M, B., C, G., A, C.C., G, M., V, V., G, K., E, G., L, B., B, M., C, F., E, D.H., G, S., A, C.C., 2021a. Biomarkers of phthalates and alternative plasticizers in the Flemish Environment and Health Study (FLEHS IV): time trends and exposure assessment. Environ. Pollut. 276 https://doi.org/10.1016/J.ENVPOL.2021.116724.

Bastiaensen, M., Gys, C., Colles, A., Verheyen, V., Koppen, G., Govarts, E., Bruckers, L., Morrens, B., Loots, I., De Decker, A., Nelen, V., Nawrot, T., De Henauw, S., Van Larebeke, N., Schoeters, G., Covaci, A., 2021b. Exposure levels, determinants and risk assessment of organophosphate flame retardants and plasticizers in adolescents (14–15 years) from the Flemish Environment and Health Study. Environ. Int. 147 https://doi.org/10.1016/j.envint.2020.106368.

Bastiaensen, M., Gys, C., Malarvannan, G., Fotache, M., Bombeke, J., Ait Bamai, Y., Araki, A., Covaci, A., 2021c. Short-term temporal variability of urinary biomarkers of organophosphate flame retardants and plasticizers. Environ. Int. 146 https://doi.org/10.1016/J.ENVINT.2020.106147.

Bastiaensen, M., Xu, F., Been, F., Van den Eede, N., Covaci, A., 2018. Simultaneous determination of 14 urinary biomarkers of exposure to organophosphate flame retardants and plasticizers by LC-MS/MS. Anal. Bioanal. Chem. 410, 7871–7880. https://doi.org/10.1007/s00216-018-1402-2.

Bocca, B., Pino, A., Brumatti, L.V., Rosolen, V., Ronfani, L., D'Aversa, J., Ruggieri, F., Petrucci, F., Calamandrei, G., Barbone, F., Alimonti, A., 2020. Children exposure to inorganic and organic arsenic metabolites: a cohort study in Northeast Italy. Environ. Pollut. 265 https://doi.org/10.1016/J.ENVPOL.2020.114826.

Bravo, N., Grimalt, J.O., Bocca, B., Pino, A., Bin, M., Brumatti, L.V., Rosolen, V., Barbone, F., Ronfani, L., Alimonti, A., Calamandrei, G., 2019. Urinary metabolites of organophosphate and pyrethroid pesticides in children from an Italian cohort (PHIME, Trieste). Environ. Res. 176 https://doi.org/10.1016/j.envres.2019.05.039.

Buekers, J., Colles, A., Cornelis, C., Morrens, B., Govarts, E., Schoeters, G., 2018. Socio-economic status and health: evaluation of human biomonitored chemical exposure to per-and polyfluorinated substances across status. Int. J. Environ. Res. Publ. Health 15. https://doi.org/10.3390/ijerph15122818.

Campanella, B., Colombaioni, L., Benedetti, E., Di Ciaula, A., Ghezzi, L., Onor, M., D'orazio, M., Giannecchini, R., Petrini, R., Bramanti, E., 2019. Toxicity of thallium at low doses: a review. Int. J. Environ. Res. Publ. Health 16. https://doi.org/10.3390/IJERPH16234732.

Černá, M., Puklová, V., Hanzlíková, L., Sochorová, L., Kubínová, R., 2017. 25 years of HBM in the Czech Republic. Int. J. Hyg Environ. Health 220, 3–5. https://doi.org/10.1016/j.ijheh.2016.08.004.

Chokwe, T.B., Abafe, O.A., Mbelu, S.P., Okonkwo, J.O., Sibali, L.L., 2020. A review of sources, fate, levels, toxicity, exposure and transformations of organophosphorus flame-retardants and plasticizers in the environment. Emerg. Contam. 6, 345–366. https://doi.org/10.1016/J.EMCON.2020.08.004.

Colles, A., Bruckers, L., Den Hond, E., Govarts, E., Morrens, B., Schettgen, T., Buekers, J., Coertjens, D., Nawrot, T., Loots, I., Nelen, V., De Henauw, S., Schoeters, G., Baeyens, W., van Larebeke, N., 2020. Perfluorinated substances in the Flemish population (Belgium): levels and determinants of variability in exposure. Chemosphere 242. https://doi.org/10.1016/j.chemosphere.2019.125250.

Colles, A., Coertjens, D., Morrens, B., Hond, E. Den, Paulussen, M., Bruckers, L., Govarts, E., Covaci, A., Koppen, G., Croes, K., Nelen, V., Van Larebeke, N., De Henauw, S., Fierens, T., Van Gestel, G., Chovanova, H., Mampaey, M., Van Campenhout, K., Loots, I., Baeyens, W., Schoeters, G., 2021. Human biomonitoring data enables evidence-informed policy to reduce internal exposure to persistent organic compounds: a case study. Int. J. Environ. Res. Publ. Health 18. https://doi.org/10.3390/ijerph18115559.

Covaci, A., Voorspoels, S., 2005. Optimization of the determination of polybrominated diphenyl ethers in human serum using solid-phase extraction and gas chromatography-electron capture negative ionization mass spectrometry. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 827, 216–223. https://doi.org/10.1016/j.jchromb.2005.09.020.

Croes, K., Den Hond, E., Bruckers, L., Govarts, E., Schoeters, G., Covaci, A., Loots, I., Morrens, B., Nelen, V., Sioen, I., Van Larebeke, N., Baeyens, W., 2015. Endocrine actions of pesticides measured in the Flemish environment and health studies (FLEHS I and II). Environ. Sci. Pollut. Control Ser. 22, 14589–14599. https://doi.org/10.1007/s11356-014-3437-z.

De Craemer, S., Croes, K., van Larebeke, N., De Henauw, S., Schoeters, G., Govarts, E., Loots, I., Nawrot, T., Nelen, V., Den Hond, E., Bruckers, L., Gao, Y., Baeyens, W., 2017. Metals, hormones and sexual maturation in Flemish adolescents in three cross-sectional studies (2002-2015). Environ. Int. 102, 190–199. https://doi.org/10.1016/j.envint.2017.02.014.

Den Hond, E., Govarts, E., Bruckers, L., Schoeters, G., 2009. Determinants of polychlorinated aromatic hydrocarbons in serum in three age classes-Methodological implications for human biomonitoring. Environ. Res. 109, 495–502. https://doi.org/10.1016/j.envres.2009.02.007.

Den Hond, E., Govarts, E., Willems, H., Smolders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castaño, A., et al., 2015. First steps toward harmonized human biomonitoring in Europe: demonstration project to perform human biomonitoring on a European scale. Environ. Health Perspect. 123 (3), 255–263. https://doi.org/10.1289/ehp.1408616.

Duffek, A., Conrad, A., Kolossa-Gehring, M., Lange, R., Rucic, E., Schulte, C., Wellmitz, J., 2020. Per- and polyfluoroalkyl substances in blood plasma – results of the German Environmental Survey for children and adolescents 2014–2017 (GerES V). Int. J. Hyg Environ. Health 228. https://doi.org/10.1016/j.ijheh.2020.113549.

ECHA, E.C., n.d. REACH Restrictions [WWW Document], URL. https://ec.europa.eu/growth/sectors/chemicals/reach/restrictions_en.

ECHA, E.C., n.d. Perfluoroalkyl chemicals [WWW Document], URL. https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas. (Accessed 12 October 2021).

European Chemical Agency (ECHA), 2018. Committee for Risk Assessment. Opinion on Scientific Evaluation of Occupational Exposure Limits for Benzene.

Eurostat, 2018. Unit E.4., R. statistics and geographical information. Methodol. Manu. Territ. Typol. https://doi.org/10.2785/228845.

Fernández, F.S., Pardo, O., Corpas-Burgos, F., Yusà, V., 2020a. Exposure and cumulative risk assessment to non-persistent pesticides in Spanish children using biomonitoring. Sci. Total Environ. 746 https://doi.org/10.1016/j.scitotenv.2020.140983.

Fernández, F.S., Pardo, O., Corpas-Burgos, F., Yusà, V., 2020b. Exposure and cumulative risk assessment to non-persistent pesticides in Spanish children using biomonitoring. Sci. Total Environ. 746 https://doi.org/10.1016/j.scitotenv.2020.140983.

FAO, 2019. FAOSTAT [WWW Document], URL. https://www.fao.org/faostat/en/#data/RP. (Accessed 12 September 2021).

Fillol, C., Oleko, A., Saoudi, A., Zeghnoun, A., Balicco, A., Gane, J., Rambaud, L., Leblanc, A., Gaudreau, É., Marchand, P., Le Bizec, B., Bouchart, V., Le Gléau, F., Durand, G., Denys, S., 2021. Exposure of the French population to bisphenols, phthalates, parabens, glycol ethers, brominated flame retardants, and perfluorinated compounds in 2014–2016: results from the Esteban study. Environ. Int. 147 https://doi.org/10.1016/j.envint.2020.106340.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds - exposure assessment for the general population in western countries. Int. J. Hyg Environ. Health. https://doi.org/10.1016/j.ijheh.2008.04.007.

Ganzleben, C., Antignac, J.P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebret, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int. J. Hyg Environ. Health 220, 94–97. https://doi.org/10.1016/J.IJHEH.2017.01.007.

Gheidarloo, M., Kelishadi, R., Hovsepian, S., Keikha, M., Hashemipour, M., 2020. The association between prenatal exposure to organochlorine compounds and neonatal thyroid hormone levels: a systematic review. J. Pediatr. Endocrinol. Metab. https://doi.org/10.1515/jpem-2019-0336.

Golub, M.S., 2000. Adolescent health and the environment. Environmental Health Perspectives 108, 355–362. https://doi.org/10.1289/ehp.00108355.

Gys, C., Ait Bamai, Y., Araki, A., Bastiaensen, M., Caballero-Casero, N., Kishi, R., Covaci, A., 2020. Biomonitoring and temporal trends of bisphenols exposure in Japanese school children. Environ. Res. 191 https://doi.org/10.1016/J.ENVRES.2020.110172.

Gys, C., Bastiaensen, M., Bruckers, L., Colles, A., Govarts, E., Martin, L.R., Verheyen, V., Koppen, G., Morrens, B., Den Hond, E., De Decker, A., Schoeters, G., Covaci, A., 2021. Determinants of exposure levels of bisphenols in flemish adolescents. Environ. Res. 193 https://doi.org/10.1016/j.envres.2020.110567.

Hahn, G.J., Meeker, W.Q., Escobar, L.A., 2016. Statistical Intervals : a Guide for Practitioners and Researchers, p. 651.

Hays, S.M., Aylward, L.L., Gagné, M., Nong, A., Krishnan, K., 2010. Biomonitoring Equivalents for inorganic arsenic. Regul. Toxicol. Pharmacol. 58, 1–9. https://doi.org/10.1016/j.yrtph.2010.06.002.

Health Canada, 2019. Fifth report on human biomonitoring of environmental chemicals in Canada - Canada.ca [WWW Document], URL. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/fifth-report-human-biomonitoring.html. (Accessed 2 February 2022).

Health Canada, 2017. Fourth report on human biomonitoring of environmental chemicals in Canada - Canada.ca [WWW Document], URL. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/fourth-report-human-biomonitoring-environmental-chemicals-canada.html. (Accessed 2 January 2022).

Iamiceli, A.L., Abate, V., Abballe, A., Bena, A., De Filippis, S.P., De Luca, S., Fulgenzi, A. R., Iacovella, N., Ingelido, A.M., Marra, V., Miniero, R., Farina, E., Gandini, M., Orengia, M., De Felip, E., 2020. Biomonitoring of the adult population in the area of turin waste incinerator: baseline levels of polycyclic aromatic hydrocarbon metabolites. Environ. Res. 181 https://doi.org/10.1016/j.envres.2019.108903.

IARC Working Group on the Evaluation of Carcinogenic Risks, 2010. Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. Iarc Monographs on the Evaluation of Carcinogenic Risks to Humans. Various.

International Agency for Research on Cancer, 2019. IARC monographs on the identification of carcinogenic hazards to humans - preamble. World Health Organ. 1–44.

Kaiser, A.M., Forsthuber, M., Aro, R., Kärrman, A., Gundacker, C., Zeisler, H., Foessleitner, P., Salzer, H., Hartmann, C., Uhl, M., Yeung, L.W.Y., 2021. Extractable organofluorine analysis in pooled human serum and placental tissue samples from an Austrian subpopulation—a mass balance analysis approach. Environ. Sci. Technol. 55, 9033–9042. https://doi.org/10.1021/acs.est.1c00883.

Kalofiri, P., Balias, G., Tekos, F., 2021. The EU endocrine disruptors' regulation and the glyphosate controversy. Toxicol Rep 8, 1193–1199. https://doi.org/10.1016/j.toxrep.2021.05.013.

Keswani, C., Dilnashin, H., Birla, H., Roy, P., Tyagi, R.K., Singh, D., Rajput, V.D., Minkina, T., Singh, S.P., 2021. Global footprints of organochlorine pesticides: a pan-global survey. Environ. Geochem. Health. https://doi.org/10.1007/s10653-021-00946-7.

Kicinski, M., Saenen, N.D., Viaene, M.K., Den Hond, E., Schoeters, G., Plusquin, M., Nelen, V., Bruckers, L., Sioen, I., Loots, I., Baeyens, W., Roels, H.A., Nawrot, T.S., 2016. Urinary t,t-muconic acid as a proxy-biomarker of car exhaust and

neurobehavioral performance in 15-year olds. Environ. Res. 151, 521–527. https://doi.org/10.1016/J.ENVRES.2016.06.035.
Kirman, C.R., Aylward, L.L., Hays, S.M., Krishnan, K., Nong, A., 2011. Biomonitoring equivalents for DDT/DDE. Regul. Toxicol. Pharmacol. 60, 172–180. https://doi.org/10.1016/j.yrtph.2011.03.012.
Koureas, M., Tsakalof, A., Tsatsakis, A., Hadjichristodoulou, C., 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol. Lett. 210, 155–168. https://doi.org/10.1016/J.TOXLET.2011.10.007.
Lamkarkach, F., Ougier, E., Garnier, R., Viau, C., Kolossa-Gehring, M., Lange, R., Apel, P., 2021. Human biomonitoring initiative (HBM4EU): human biomonitoring guidance values (HBM-GVs) derived for cadmium and its compounds. Environ. Int. 147 https://doi.org/10.1016/j.envint.2020.106337.
Lange, R., Apel, P., Rousselle, C., Charles, S., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2021a. The European Human Biomonitoring Initiative (HBM4EU): human biomonitoring guidance values for selected phthalates and a substitute plasticizer. Int. J. Hyg Environ. Health 234. https://doi.org/10.1016/j.ijheh.2021.113722.
Lange, R., Apel, P., Rousselle, C., Charles, S., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2021b. The European Human Biomonitoring Initiative (HBM4EU): human biomonitoring guidance values for selected phthalates and a substitute plasticizer. Int. J. Hyg Environ. Health 234, 113722. https://doi.org/10.1016/j.ijheh.2021.113722.
Lebelo, K., Malebo, N., Mochane, M.J., Masinde, M., 2021. Chemical contamination pathways and the food safety implications along the various stages of food production: a review. Int. J. Environ. Res. Publ. Health. https://doi.org/10.3390/ijerph18115795.
Lemke, N., Murawski, A., Schmied-Tobies, M.I.H., Rucic, E., Hoppe, H.W., Conrad, A., Kolossa-Gehring, M., 2021. Glyphosate and aminomethylphosphonic acid (AMPA) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). Environ. Int. 156, 106769 https://doi.org/10.1016/j.envint.2021.106769.
Lubin, J.H., Colt, J.S., Camann, D., Davis, S., Cerhan, J.R., Severson, R.K., Bernstein, L., Hartge, P., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ. Health Perspect. 112, 1691–1696. https://doi.org/10.1289/EHP.7199.
Luis, C., Algarra, M., Camara, J., Perestrelo, R., 2021. Comprehensive insight from phthalates occurrence: from health outcomes to emerging analytical approaches. Toxics 9 (7), 157. https://doi.org/10.3390/toxics9070157.
Montazeri, P., Thomsen, C., Casas, M., de Bont, J., Haug, L.S., Maitre, L., Papadopoulou, E., Sakhi, A.K., Slama, R., Saulnier, P.J., Urquiza, J., Grazuleviciene, R., Andrusaityte, S., McEachan, R., Wright, J., Chatzi, L., Basagaña, X., Vrijheid, M., 2019. Socioeconomic position and exposure to multiple environmental chemical contaminants in six European mother-child cohorts. Int. J. Hyg Environ. Health 222, 864–872. https://doi.org/10.1016/j.ijheh.2019.04.002.
Monteiro De Oliveira, E.C., Caixeta, E.S., Santos, V.S.V., Pereira, B.B., 2021. Arsenic exposure from groundwater: environmental contamination, human health effects, and sustainable solutions. J. Toxicol. Environ. Health B Crit. Rev. https://doi.org/10.1080/10937404.2021.1898504.
Morgan, M.K., 2012. Children's exposures to pyrethroid insecticides at home: a review of data collected in published exposure measurement studies conducted in the United States. Int. J. Environ. Res. Publ. Health. https://doi.org/10.3390/ijerph9082964.
Morrens, B., Bruckers, L., Hond, E. Den, Nelen, V., Schoeters, G., Baeyens, W., Van Larebeke, N., Keune, H., Bilau, M., Loots, I., 2012. Social distribution of internal exposure to environmental pollution in Flemish adolescents. Int. J. Hyg Environ. Health 215, 474–481. https://doi.org/10.1016/j.ijheh.2011.10.008.
Morrens, B., Den Hond, E., Schoeters, G., Coertjens, D., Colles, A., Nawrot, T.S., Baeyens, W., De Henauw, S., Nelen, V., Loots, I., 2017. Human biomonitoring from an environmental justice perspective: supporting study participation of women of Turkish and Moroccan descent. Environ. Health: Glob. Access Sci. Source 16, 1–9. https://doi.org/10.1186/s12940-017-0260-2.
Murawski, A., Roth, A., Schwedler, G., Schmied-Tobies, M.I.H., Rucic, E., Pluym, N., Scherer, M., Scherer, G., Conrad, A., Kolossa-Gehring, M., 2020. Polycyclic aromatic hydrocarbons (PAH) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). Int. J. Hyg Environ. Health 226, 113491. https://doi.org/10.1016/J.IJHEH.2020.113491.
Norén, E., Lindh, C., Rylander, L., Glynn, A., Axelsson, J., Littorin, M., Faniband, M., Larsson, E., Nielsen, C., 2020. Concentrations and temporal trends in pesticide biomarkers in urine of Swedish adolescents, 2000–2017. J. Expo. Sci. Environ. Epidemiol. 30, 756–767. https://doi.org/10.1038/s41370-020-0212-8.
Pearson, M., Lu, C., Schmotzer, B., Waller, L., Riederer, A., 2009. Evaluation of physiological measures for correcting variation in urinary output: Implications for assessing environmental chemical exposure in children. J. Expo. Sci. Environ. Epidemiol. 19 (3), 336–342. https://doi.org/10.1038/jes.2008.48.
Pirard, C., Remy, S., Giusti, A., Champon, L., Charlier, C., 2020. Assessment of children's exposure to currently used pesticides in wallonia, Belgium. Toxicol. Lett. 329, 1–11. https://doi.org/10.1016/j.toxlet.2020.04.020.
Rappazzo, K.M., Coffman, E., Hines, E.P., 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. Int. J. Environ. Res. Publ. Health. https://doi.org/10.3390/ijerph14070691.
Rodzaj, W., Wileńska, M., Klimowska, A., Dziewirska, E., Jurewicz, J., Walczak-Jędrzejowska, R., Słowikowska-Hilczer, J., Hanke, W., Wielgomas, B., 2021. Concentrations of urinary biomarkers and predictors of exposure to pyrethroid insecticides in young, Polish, urban-dwelling men. Sci. Total Environ. 773 https://doi.org/10.1016/j.scitotenv.2021.145666.
Roelants, M., Hauspie, R., Hoppenbrouwers, K., 2009. References for growth and pubertal development from birth to 21 years in Flanders, Belgium. Ann. Hum. Biol. 36, 680–694. https://doi.org/10.3109/03014460903049074.
Saillenfait, A.M., Ndiaye, D., Sabaté, J.P., 2015. Pyrethroids: exposure and health effects - an update. Int. J. Hyg Environ. Health. https://doi.org/10.1016/j.ijheh.2015.01.002.
Schoeters, G., Govarts, E., Bruckers, L., Den Hond, E., Nelen, V., De Henauw, S., Sioen, I., Nawrot, T.S., Plusquin, M., Vriens, A., Covaci, A., Loots, I., Morrens, B., Coertjens, D., Van Larebeke, N., De Craemer, S., Croes, K., Lambrechts, N., Colles, A., Baeyens, W., 2017. Three cycles of human biomonitoring in Flanders – time trends observed in the flemish environment and health study. Int. J. Hyg Environ. Health 220, 36–45. https://doi.org/10.1016/J.IJHEH.2016.11.006.
Schoeters, G., Hond, E. Den, Colles, A., Loots, I., Morrens, B., Keune, H., Bruckers, L., Nawrot, T., Sioen, I., De Coster, S., Van Larebeke, N., Nelen, V., Van de Mieroop, E., Vrijens, J., Croes, K., Goeyens, K., Baeyens, W., 2012. Concept of the Flemish human biomonitoring programme. Int. J. Hyg Environ. Health 215, 102–108. https://doi.org/10.1016/J.IJHEH.2011.11.006.
Schrenk, D., Bignami, M., Bodin, L., Chipman, J.K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Leblanc, J.C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregård, L., Ceccatelli, S., Cravedi, J.P., Halldorsson, T.I., Haug, L.S., Johansson, N., Knutsen, H.K., Rose, M., Roudot, A.C., Van Loveren, H., Vollmer, G., Mackay, K., Riolo, F., Schwerdtle, T., 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. 18, e06223 https://doi.org/10.2903/j.efsa.2020.6223.
Schroijen, C., Baeyens, W., Schoeters, G., Den Hond, E., Koppen, G., Bruckers, L., Nelen, V., Van De Mieroop, E., Bilau, M., Covaci, A., Keune, H., Loots, I., Kleinjans, J., Dhooge, W., Van Larebeke, N., 2008. Internal exposure to pollutants measured in blood and urine of Flemish adolescents in function of area of residence. Chemosphere 71, 1317–1325. https://doi.org/10.1016/j.chemosphere.2007.11.053.
Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2011. Update of the reference and HBM values derived by the German human biomonitoring commission. Int. J. Hyg Environ. Health 215, 26–35. https://doi.org/10.1016/j.ijheh.2011.06.007.
Schwedler, G., Rucic, E., Lange, R., Conrad, A., Koch, H.M., Pälmke, C., Brüning, T., Schulz, C., Schmied-Tobies, M.I.H., Daniels, A., Kolossa-Gehring, M., 2020. Phthalate metabolites in urine of children and adolescents in Germany. Human biomonitoring results of the German Environmental Survey GerES V, 2014–2017. Int. J. Hyg Environ. Health 225. https://doi.org/10.1016/J.IJHEH.2019.113444.
n.d. Scientific opinion on lead in food | European food safety authority [WWW Document], URL. https://www.efsa.europa.eu/en/efsajournal/pub/1570, 8.19.21.
Sochacka-Tatara, E., Majewska, R., Perera, F.P., Camann, D., Spengler, J., Wheelock, K., Sowa, A., Jacek, R., Mróz, E., Pac, A., 2018. Urinary polycyclic aromatic hydrocarbon metabolites among 3-year-old children from Krakow, Poland. Environ. Res. 164, 212–220. https://doi.org/10.1016/j.envres.2018.02.032.
Stajnko, A., Snoj Tratnik, J., Kosjek, T., Mazej, D., Jagodic, M., Eržen, I., Horvat, M., 2020. Seasonal glyphosate and AMPA levels in urine of children and adolescents living in rural regions of Northeastern Slovenia. Environ. Int. 143 https://doi.org/10.1016/J.ENVINT.2020.105985/SEASONAL_GLYPHOSATE_AND_AMPA_LEVELS_IN_URINE_OF_CHILDREN_AND_ADOLESCENTS_LIVING_IN_RURAL_REGIONS_OF_NORTHEASTERN_SLOVENIA.PDF.
Tschersich, C., Murawski, A., Schwedler, G., Rucic, E., Moos, R.K., Kasper-Sonnenberg, M., Koch, H.M., Brüning, T., Kolossa-Gehring, M., 2021. Bisphenol A and six other environmental phenols in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). Sci. Total Environ. 763 https://doi.org/10.1016/j.scitotenv.2020.144615.
Tyrrell, J., Melzer, D., Henley, W., Galloway, T.S., Osborne, N.J., 2013. Associations between socioeconomic status and environmental toxicant concentrations in adults in the USA: NHANES 2001-2010. Environ. Int. 59, 328–335. https://doi.org/10.1016/j.envint.2013.06.017.
US-Centers for Disease Control and Prevention, n.d. National environmental public health tracking network query tool, National report on human exposure to environmental chemicals [WWW Document], URL. https://ephtracking.cdc.gov/DataExplorer/. (Accessed 12 September 2021).
Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–177. https://doi.org/10.1016/J.REPROTOX.2007.07.010.
Verheyen, V.J., Remy, S., Govarts, E., Colles, A., Rodriguez Martin, L., Koppen, G., Voorspoels, S., Bruckers, L., Bijnens, E.M., Vos, S., Morrens, B., Coertjens, D., De Decker, A., Franken, C., Den Hond, E., Nelen, V., Covaci, A., Loots, I., De Henauw, S., Van Larebeke, N., Teughels, C., Nawrot, T.S., Schoeters, G., 2021. Urinary polycyclic aromatic hydrocarbon metabolites are associated with biomarkers of chronic endocrine stress, oxidative stress, and inflammation in adolescents: FLEHS-4 (2016-2020). Toxics 9. https://doi.org/10.3390/TOXICS9100245.
VITO, 2021. EU HBM Dashboard – HBM4EU – science and policy for a healthy future [WWW Document], URL. https://www.hbm4eu.eu/what-we-do/european-hbm-platform/eu-hbm-dashboard/. (Accessed 12 September 2021).
Vlaamse Milieumaatschappij, 2020. Uitstoot en luchtkwaliteit in Vlaanderen- evaluatie 2020.
Vlaamse Milieumaatschappij, 2017. Air quality and emissions in the Flanders region [WWW Document], URL. https://en.vmm.be/publications/annual-report-air-quality-in-the-flanders-region-2017.
Vogel, N., Murawski, A., Schmied-Tobies, M.I.H., Rucic, E., Doyle, U., Kämpfe, A., Höra, C., Hildebrand, J., Schäfer, M., Drexler, H., Göen, T., Kolossa-Gehring, M., 2021. Lead, cadmium, mercury, and chromium in urine and blood of children and adolescents in Germany – human biomonitoring results of the German

Environmental Survey 2014–2017 (GerES V). Int. J. Hyg Environ. Health 237, 113822. https://doi.org/10.1016/J.IJHEH.2021.113822.
Wei, B., Richard O'connor, Goniewicz, M., Hyland, Andrew, 2020. Association between urinary metabolite levels of organophosphorus flame retardants and serum sex hormone levels measured in a reference sample of the US general population. https://doi.org/10.1007/s12403-020-00353-w.
Wilhelm, M., 2014. Human biomonitoring. Its importance in toxicological regulation. Regul. Toxicol. 685–702. https://doi.org/10.1007/978-3-642-35374-1_84.