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Investigating children's chemical exposome – Description and possible determinants of exposure in the region of Luxembourg based on hair analysis

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ABSTRACT

The specific physiology and behaviour of children makes them particularly vulnerable to chemical exposure. Specific studies must therefore be conducted to understand the impact of pollution on children's health. Human biomonitoring is a reliable approach for exposure assessment, and hair, allowing the detection of parent chemicals and metabolites, and covering wider time windows than urine and blood is particularly adapted to study chronic exposure. The present study aims at assessing chemical exposure and investigating possible determinants of exposure in children living in Luxembourg.

Hair samples were collected from 256 children below 13 y/o and tested for 153 compounds (140 pesticides, 4 PCBs, 7 BDEs and 2 bisphenols). Moreover, anthropometric parameters, information on diet, residence, and presence of pets at home was collected through questionnaires. Correlations, regressions, t-tests, PLS-DA and MANOVAs, were used to investigate exposure patterns.

Twenty-nine to 88 (median = 61) compounds were detected per sample. The highest median concentration was observed for BPA (133.6 pg/mg). Twenty-three biomarkers were detected in ≥ 95% of the samples, including 13 in all samples (11 pesticides, BPA and BPS). Exposure was higher at younger ages ($R^2 = 0.57$), and boys were more exposed to non-persistent pesticides than girls. Presence of persistent organic pollutants in most children suggests that exposure is still ongoing. Moreover, diet (e.g. imazalil: 0.33 pg/mg in organic, 1.15 pg/mg in conventional, p-value < 0.001), residence area (e.g. imidacloprid: 0.29 pg/mg in urban, 0.47 pg/mg in countryside, p-value = 0.03), and having pets (e.g. fipronil: 0.32 pg/mg in pets, 0.09 pg/mg in no pets, p-value < 0.001) were identified as determinants of exposure.

The present study demonstrates that children are simultaneously exposed to multiple pollutants from different chemical classes, and confirms the suitability of hair to investigate exposure. These results set the basis for further investigations to better understand the determinants of chemical exposure in children.

1. Introduction

Exposure to chemical pollutants at young ages has been associated with severe health effects in children such as neurological diseases, developmental issues, endocrine disruption, respiratory and cardiovascular disorders, or child cancer (Garry 2004; Makri et al. 2004; Predieri et al. 2021; Raherison et al. 2019; Roth and Wilks 2014; Sanders et al. 2018; Vrijheid et al. 2016). Particularly, an increasing amount of epidemiological studies has already advised on the environmental causes of certain disorders. For instance, exposure to pesticides has been associated with obesity in children and adolescents, and other cardiovascular issues such as high blood pressure and overweight (Buser et al. 2014; Parastar et al. 2018; Twum and Wei 2011). Pesticides have also been associated with hormonal changes and delay in sexual and puberty

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development in adolescents (Den Hond and Schoeters 2006; Ozen et al. 2014). Chemical exposure to different classes has been also associated with neurodevelopmental disorders, mental diseases and changes on the brain structure (Budtz-Jørgensen et al. 2013; Engel et al. 2018; Rauh et al. 2011). Additionally, exposure to pollutants have been also associated with cancer development in children, and epidemiological studies found associations between exposure to pesticides and leukaemia, which is the most common cancer during childhood (Belson et al. 2007; Hernández and Menéndez 2016; Turner et al. 2010). In 2018, the Lancet Commission on Exposure and Health stated that from all deaths of children younger than 5 years old, a quarter is caused by exposure to unhealthy environment, in particular to pollution caused by "industrial emissions, vehicular exhausts, and toxic chemicals" (Landrigan et al. 2018). On top of that, the Commission also highlighted that globally, approximately one third of the life years of children under the age of 10 are lost due to illness, poor health and disability due to pollution-related pathologies, which could lead to early death (Landrigan et al. 2018).

The effects of chemical exposure in children could strongly differ from the ones in adults because of their behavioural and physiological differences. Indeed, although children are smaller, their surface-to-volume ratio is three times higher than in adults, they have periods of fast growth and they tend to consume more food per unit of weight than adults (Jackson 1994; Landrigan et al. 2004; Landrigan et al. 2018). Children have faster metabolism, their breath rate is higher than in adults, and their organ system is in continuous development (Landrigan et al. 2004; Landrigan et al. 2018). As a result, children exposure cannot be directly extrapolated from data obtained from adults, and only studies conducted specifically on children can provide relevant information. Nevertheless, most epidemiological studies investigating exposure to pollutants were conducted on adult populations, and although epidemiological studies investigating children exposure have increased, data on children remain more limited, particularly when exposure assessment is based on hair analysis.

Human biomonitoring comes across as the best approach to assess exposure. Indeed, measuring pollutant concentration in biological matrices provides information that integrates all the possible sources of exposure, and is specific to the subject the sample have been collected from. In this field, hair was demonstrated to be one of the best matrices to assess exposure, due to the many advantages it provides (Appenzeller et al. 2017; Appenzeller and Tsatsakis 2012; Hardy et al. 2021; Peng et al. 2021). Compared to fluids in which only parent chemicals (for blood) or hydrophilic metabolites (in urine) can usually be detected, hair is suitable to assess exposure to many different families, including parent compounds and metabolites of both persistent and non-persistent organic pollutants (Appenzeller and Tsatsakis 2012; Iglesias-González et al. 2021). Moreover, contrary to blood and urine, which only give information on short term exposure and present high variability in pollutant concentration (often covering several orders of magnitude within a few hours), hair is representative of wider time windows, covering up to several months depending on hair length (Appenzeller and Tsatsakis 2012; Faÿs et al. 2020; Peng et al. 2021). In this regard, a recent article demonstrated that whereas a 6 cm-long hair sample provides information integrating exposure over 6 months, approximately 50 urine samples collected over the same period only represent 3–4% of the total volume of urine produced over the same period (Fäys et al. 2021). Hair therefore appears as the best suited matrix for chronic exposure assessment, and has gained increasing attention for this purpose in epidemiological studies.

The present study was conducted to assess the exposure to pollutants in a group of 256 children living in Luxembourg, and to investigate possible sources of exposure. This study is the first one investigating children chemical exposome and determinants of exposure by means of hair analysis. The results should enable to better understand children chemical exposome, and help to define more efficient prevention measures specifically targeted to children.

2. Material and methods

2.1. Description of the area: Luxembourg

The Grand Duchy of Luxembourg is a country situated in Western Europe, bordered by Germany, France and Belgium, and it is one of the four capitals of the European Union. Luxembourg has an area of 2,595 km2, and counted 626.108 inhabitants in June 2021, making it one of the most densely populated countries in Europe (European Commission 2016). Nowadays, Luxembourg's economy is led by the financial sector, which accounts for more than 35% of the Country's economy, while industry represents just 6% and agriculture 0.3% (Central Inteligence Agency 2021; Luxinnovation, 2019). However, Luxembourg has a strong industrial past, led by the increase of the metal industry in parallel to the industrial revolution, and until the 70′s, industry contributed to more than 40% of the Country's economy (Chambre of Comerce Luxembourg 2015). The industrial history of the country could be responsible for environmental contamination with persistent organic pollutants (POPs) and subsequent exposure of the population, as suggested in a previous study conducted on adults (Peng et al. 2021).

2.2. Hair sample and information collection

A total of 264 children inhabitants of Luxembourg took part in the present study. The only selection criteria were to be aged 13 or below, and to live in the Grand Duchy of Luxembourg. Information concerning anthropometric parameters, diet, residence area and presence of pets at home was collected through questionnaires completed by their parents/tutors. The recruitment of participants was conducted from October 2020 to April 2021. Hair samples were collected by the parents at home after contacting the laboratory to request the sampling kit. Each sampling kit included a detailed sampling protocol with pictures of each step, an inform consent to be signed by the parents, a questionnaire and a pre-payed envelop to send the samples, the questionnaire and the signed inform consent back to the laboratory. Hair was cut at the vertex region of the scalp, closest to the skin as possible, and placed in aluminium foil. The study was approved by the Luxembourg ethic committee (Comité national d'éthique de recherche N° 201911/01).

2.3. Sample decontamination and preparation

Prior to the chemical analysis, samples were measured and cut at the desired length. Sample length was limited to a maximum of 9 cm starting from the skin, since previous studies demonstrated that no significant effect of hair length on chemical concentration was observed until this level (Beranger et al. 2018). Most samples (n = 138) were between 6 cm and 9 cm long and only 79 samples were shorter. The fact that using different lengths corresponds to different time period is not considered a problem for this specific study since we did not investigate exposure during a specific period of the year. Prior to chemical extraction, each sample was washed with three successive baths of a) SDS (Sodium dodecyl sulphate from Sigma-Aldrich – ReagentPlus*L4509), b) ultrapure water (Millipore-AFS-8 system) and c) methanol (Biosolve-Analytical grade) under agitation, following the validated protocol described in Duca et al. (2014). Then, samples were put in a paper tissue, gently dabbed, and placed under soft airflow to dry. Once the samples were dry, they were placed in a stainless grind jar for pulverization using a ball mill Retsch MM200 at 25 Hz and 50 mg of hair powder per sample were placed in a 4 ml screw neck glass vials (La-Pha-Pack).

Eight hair samples did not have enough material to be analysed, and therefore, they were not included in this study.

2.4. Chemical extraction and analysis

The methodology used in the chemical analysis explained below has been fully validated and published in Hardy et al. (2015) and Beranger

et al. (2018). Each sample was supplemented with 10 μl of internal standard solution (stable isotope labelled analogues from Dr. Ehrenstorfer, Sigma-Aldrich, Toronto Research Chemicals (Toronto, ON, Canada), Cambridge Isotope laboratories (Tewksbury, MA, USA) and US Biological (Swampscott, MA, USA)), and 1 ml of a mixture of acetonitrile and water (Biosolve-ULC/MS grade), and placed in a New Brunswick-G25 incubator shaker at 37 °C under agitation at 350 spm during 12 h. After 12 h extraction, the samples were centrifuged during 10 min at 2800g (Sigma 4–16 KS). The supernatant was then split to follow three different analyses:

1) To analyse non-persistent organic pollutants, 200 μl of the recovered extract were evaporated under soft nitrogen stream flow and reconstituted in 50 μl of a solution of acetonitrile and ammonium acetate buffer 10 mM. Samples were centrifuged during 5 min at 18000xg. (Centrifuge Sigma 1–16 K). Supernatants were recovered and placed in an injection vial (screw neck vials 2 ml, La-Pha-Pack) and placed for injection on a LC-MS/MS equipment (Atlantis, Waters).
2) To analyse persistent organic pollutants, 300 μl of extract were placed in 10 ml screw neck glass vial with metal caps (Supelco®) with 7.6 ml of phosphate buffer 1 M. Samples were placed on a GC–MS/MS (Agilent Technologies 7000A model) to perform a solid phase micro extraction (SPME).
3) Persistent organic pollutants were also analysed by liquid injection on a GC–MS/MS. For this, 300 μl were placed in a 5 ml screw vials (La-Pha-Pack) and evaporated under soft nitrogen stream flow. After evaporation, there was added 100 ml of a mix of acetonitrile (Biosolve-ULC/MS grade) and PFBBr (2, 3, 4, 5, 6-pentafluorobenzyl bromide, Sigma Aldrich-101052). The mixture was heated during 30 min and the liquid phase was placed in a 6 ml evaporation tube (Corning-PYREX®) and evaporated to dryness. The extract was reconstituted in 200 μl of ethyl acetate (Biosolve-AR) and centrifuged at 18000 × g. for 5 min (Centrifuge Sigma 1–16 K). The supernatant was placed in an injection vial (screw neck vials 2 ml, La-Pha-Pack) and evaporated to be reconstituted in 20 μl of ethyl acetate (Biosolve-AR) and injected in GC–MS/MS.

Nine different quality controls were added to each analytical run, and analysed along with the samples. Quality controls consisted in one matrix blank and eight controls supplemented at different concentrations: 0.5, 1, 2, 5, 10, 20, 50 and 100 pg/mg.

2.5. Statistical analysis

Raw data was used to calculate percentage of detection, percentiles, limit of detection and maximum values. These calculations were done on Microsoft Excel version 2013. For the rest of statistical analysis mentioned in this study, we used biomarkers with a detection frequency (DF) ≥ 50%, concentration values were log transformed, and non-detected (nd) values were substituted by censored multiple imputation in RStudio version 1.3.1093 using the R package "EnvStats". Spearman and Pearson correlations, and Mann-Whitney tests, were done using Systat Software, Inc. Sigma Plot for Windows version 12.5 to study the associations between exposure and age, and to highlight the differences in exposure between groups. Partial least squares-discriminant analysis (PLS-DA) was used to illustrate the identification of clusters in the population regarding their diet and residence area. PLS-DA was performed in RStudio version 1.3.1093 using the R package "mixOmics" and the function "plsda". Multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) were done in RStudio version 1.3.1093 using the R package "dplyr". MANOVA and ANOVA were used to investigate if the differences in exposure between groups (taking in consideration all biomarkers detected ≥ 50%) were significant. Multiple and single regressions were done in RStudio version 1.3.1093 using the R package "stats". Boxplots were done using RStudio version 1.3.1093 using the package "ggplot2".

3. Results

Associations between exposure and the different variables presented in this study were tested using biomarkers with a detection frequency (DF) ≥ 50% (Table 2). The associations of pollutants concentration with age and sex was therefore tested on 59 biomarkers. Since information on diet, residence area and pets was not available for 39 children, the association between these parameters and exposure was only tested for the biomarkers detected in ≥ 50% in the children for whom the information was available, therefore, 217 children instead of 256 (Table 1). PLS-DA models and regressions were adjusted for age and sex.

3.1. Biomarkers' frequency of detection and concentration

The number of biomarkers detected per child ranged from 29 to 88 biomarkers (avg. = 60.5, median = 61). Among the 153 biomarkers tested, 26 were detected in 95% of the participants or more, including 13 biomarkers detected in all the children (11 pesticides, BPA and BPS). Fifteen biomarkers were detected in 75% to 95%. Eighteen biomarkers were detected in 50% to 75% of the samples. Thirty-five biomarkers were detected in 10% to 50%, and 42 biomarkers were detected in less than 10% of the samples. Seventeen biomarkers were not detected in any of the samples analysed. Bisphenol A presented both the highest concentration (35856 pg/mg) and the highest median concentration (133.6 pg/mg) (Table 2). A heat map showing the correlations between biomarkers is available in the supplemental material (figure SM1).

3.2. Influence of age and sex on biomarker's concentration

Among the 59 biomarkers included in this statistical analysis (DF > 50%), age was significantly associated with 38 biomarkers, and all the regressions and correlations observed were negative but for lenacil (Table 3). Moreover, the relation between age and concentration levels was also confirmed by a multiple regression obtaining a $R^2 = 0.57$ (p-value = <2.2E-16).

Sex was significantly associated with 28 biomarkers, which showed significant differences between boys and girls. Nineteen biomarkers (all

Table 1
Description of the population

Number of children	
Participants included in the present study	256
Number of girls	116
Number of boys	140
Information regarding BMI:	
Number of children who provided anthropometric parameters	209
• BMI of children ≤2 years old ranged from 13.10 to 18.48	34
• BMI of children aged between 2 to 5 years old ranged from 12.95 to 20.07	61
• BMI of children aged between 5 to 13 years old ranged from 11.72 to 31	114
• Diet:	
• Children who provided information on their diet	217
• Children following organic diet	61
• Children following traditional diet	156
• Missing data regarding diet	39
• Residence area:	
• Children who provided information on the area where they live	217
• Children living in the country side	94
• Children living in urban areas	123
• Missing data regarding residence area	39
• Pets	
• Children who provided information on their pets and use of pest control products	217
• Children who have pets	86
• Children who do not have pets	131
• Children using pest control products (only children with pets)	47
• Children not using pest control products (only children with pets)	39
• Missing data regarding pets	39

Table 2
Descriptive summary of concentrations detected in the children's hair samples, organized by chemical classes.

Compound	LOD (pg/mg)	Detection frequency (%)	25% Percentile (pg/mg)	50% Percentile (pg/mg)	75% Percentile (pg/mg)	95% Percentile (pg/mg)	Highest value (pg/mg)
Organochlorines							
α-HCH	0.034	3.13	<0.034	<0.034	<0.034	<0.034	0.232
β-HCH	0.127	4.29	<0.127	<0.127	<0.127	<0.127	1.832
ɣ-HCH	0.009	96.5	0.123	0.257	0.598	2.452	26.09
ξ-HCH	–	–	–	–	–	–	–
Ɛ-HCH	–	–	–	–	–	–	–
o,p'-DDE	–	–	–	–	–	–	–
p,p'-DDE	0.297	21.5	<0.297	<0.297	<0.297	3.029	6.281
o,p'-DDD	0.957	0.39	<0.957	<0.957	<0.957	<0.957	0.957
p,p'-DDD	–	–	–	–	–	–	–
o,p'-DDT	–	–	–	–	–	–	–
p,p'-DDT	1.795	2.73	<1.795	<1.795	<1.795	<1.795	10.42
Aldrin	0.023	10.9	<0.023	<0.023	<0.023	1.278	3.156
Isodrin	0.116	0.78	<0.116	<0.116	<0.116	<0.116	0.580
Dieldrin	0.019	17.2	<0.019	<0.019	<0.019	0.171	5.755
Endrin	–	–	–	–	–	–	–
α-Endosulfan	0.002	64.8	<0.002	0.011	0.034	0.151	0.722
β-Endosulfan	0.005	19.1	<0.005	<0.005	<0.005	0.167	1.051
Heptachlor	0.012	40.6	<0.012	<0.012	0.193	0.613	11.55
Heptachlor-*exo*-epoxide	0.382	0.39	<0.382	<0.382	<0.382	<0.382	0.382
Heptachlor-*endo*-epoxide	–	–	–	–	–	–	–
Trans-chlordane	0.012	4.69	<0.012	<0.012	<0.012	<0.012	0.068
Cis-chlordane	–	–	–	–	–	–	–
Oxy-chlordane	0.069	0.78	<0.069	<0.069	<0.069	<0.069	0.118
Pentachlorophenol (PCP)	0.150	99.6	0.925	2.103	5.119	29.32	189.7
Hexachlorobenzene (HCB)	0.019	100	0.065	0.091	0.127	0.225	1.372
Metazachlor	0.003	30.9	<0.003	<0.003	0.009	0.050	0.153
Oroganophosphates							
Dimethoate	0.003	5.08	<0.003	<0.003	<0.003	0.001	0.065
2-isopropyl-6-methyl-4-pyrimidinol (IMPy)	0.008	58.6	<0.008	0.031	0.065	0.262	2.127
DMP	0.013	91.8	0.135	0.260	0.598	2.172	240.5
DMTP	0.0003	69.9	<0.0003	0.005	0.017	0.075	910.9
DMDTP	0.110	6.64	<0.110	<0.110	<0.110	0.171	1056.0
DEP	0.221	99.6	0.777	1.332	2.382	7.780	32.13
DETP	0.026	99.6	0.075	0.137	0.253	2.025	18.79
DEDTP	0.076	0.78	<0.076	<0.076	<0.076	<0.076	0.136
TCPy	0.030	100	0.221	0.404	0.783	1.865	5.245
P-Nitrophenol (PNP)	2.253	100	5.642	8.157	10.83	16.37	69.16
3Me4NP	0.052	98.8	0.341	0.578	0.949	1.701	2.779
Malathion	0.045	63.3	<0.045	0.433	2.044	8.334	128.9
Pyrethroids							
Bifenthrin	0.022	40.6	<0.022	<0.022	0.186	0.685	3.309
Cyhalothrin	0.004	43.4	<0.004	<0.004	0.082	0.357	217.5
Permethrin	0.161	97.7	6.108	15.543	49.405	202.424	29822.2
Cyfluthrin	0.097	3.13	<0.097	<0.097	<0.097	<0.097	1.719
Cypermethrin	0.052	39.1	<0.052	<0.052	0.294	2.880	25.990
Fenvalerate	–	–	–	–	–	–	–
Deltamethrin	0.305	5.86	<0.305	<0.305	<0.305	0.340	1.823
2-ClBA	0.020	4.29	<0.020	<0.020	<0.020	0.000	0.575
Br_2CA	0.003	14.4	<0.003	<0.003	<0.003	0.042	0.282
Trans-Cl_2CA	0.099	99.2	0.428	0.693	1.487	10.42	104.4
$ClCF_3CA$	0.0005	89.8	0.016	0.055	0.134	0.288	16.79
4F3PBA	0.002	85.6	0.006	0.013	0.034	0.184	1.879
3-PBA	0.107	99.2	0.300	0.510	1.188	4.467	36.61
PCBs							
PCB 101	0.075	2.73	<0.075	<0.075	<0.075	<0.075	3.806
PCB 153	0.041	51.2	<0.041	0.054	0.306	1.181	8.355
PCB 138	0.078	43.4	<0.078	<0.078	0.379	1.335	8.765
PCB 180	0.007	93.8	0.061	0.123	0.198	0.504	8.977
Azoles							
Bitertanol	0.108	0.78	<0.108	<0.108	<0.108	<0.108	0.150
Cyproconazole	0.011	7.03	<0.011	<0.011	<0.011	0.019	0.415
Difenoconazole	0.006	63.7	<0.006	0.017	0.044	0.122	0.806
Epoxiconazole	0.058	17.9	<0.058	<0.058	<0.058	0.255	0.877
Fenbuconazole	0.073	7.42	<0.073	<0.073	<0.073	0.163	3.586
Flusilazole	0.0001	9.77	<0.0001	<0.0001	<0.0001	0.005	0.334
Imazalil	0.168	54.7	<0.168	0.729	2.546	11.22	35.12
Myclobutanil	0.004	46.9	<0.004	<0.004	0.039	0.192	1.716
Penconazole	0.038	17.6	<0.038	<0.038	<0.038	0.158	0.725
Prochloraz	0.0004	33.6	<0.0004	<0.0004	0.009	0.149	0.950
Propiconazole	0.030	91.4	0.224	0.579	1.443	7.523	40.27
Tebuconazole	0.024	91.0	0.155	0.272	0.505	2.963	17.06

(*continued on next page*)

Table 2 (*continued*)

Compound	LOD (pg/mg)	Detection frequency (%)	25% Percentile (pg/mg)	50% Percentile (pg/mg)	75% Percentile (pg/mg)	95% Percentile (pg/mg)	Highest value (pg/mg)
Tetraconazole	0.062	21.1	<0.062	<0.062	<0.062	0.404	1.580
Thiabendazole	0.059	100	0.602	1.412	4.192	17.22	112.6
Triadimenol	0.194	2.34	<0.194	<0.194	<0.194	<0.194	1.022
Strobilurins							
Azoxystrobin	0.016	98.1	0.218	0.625	1.919	9.896	138.1
Kresoxim-methyl	0.369	0.39	<0.369	<0.369	<0.369	<0.369	0.369
Pyraclostrobin	0.006	77.3	0.009	0.029	0.076	0.216	1.095
Trifloxystrobin	0.002	87.9	0.007	0.016	0.045	0.241	4.274
Acid Herbicides							
Mecroprop	0.059	95.7	0.248	0.437	0.735	1.512	8.515
MCPA	0.044	99.2	0.168	0.437	1.317	2.816	35.71
Dichlorprop	0.007	18.4	<0.007	<0.007	<0.007	0.104	0.850
2,4-D	0.045	100	0.140	0.233	0.446	5.338	124.1
MCPB	0.004	23.4	<0.004	<0.004	<0.004	0.059	0.452
2,4-DB	0.001	50.8	<0.001	0.001	0.030	0.064	0.298
Anilino-pyrimidines							
Pyrimethanil	0.019	61.7	< 0.019	0.131	0.389	1.710	29.30
Cyprodinil	0.072	17.6	< 0.072	< 0.072	< 0.072	0.352	122.6
BDEs							
BDEs 28 + 33	–	–	–	–	–	–	–
BDE 47	0.097	50.4	< 0.097	0.120	0.382	1.105	10.69
BDE 100	0.045	42.6	< 0.045	< 0.045	0.207	0.595	2.618
BDE 99	0.344	7.42	< 0.344	< 0.344	< 0.344	0.779	6.917
BDE 154	0.062	5.86	< 0.062	< 0.062	< 0.062	0.083	0.376
BDE 153	0.055	1.56	< 0.055	< 0.055	< 0.055	< 0.055	3.052
Benzamides							
Zoxamide	0.006	17.6	< 0.006	< 0.006	< 0.006	0.033	0.139
Propyzamide	0.163	1.17	< 0.163	< 0.163	< 0.163	< 0.163	0.270
Dinitroanilines							
Trifluralin	0.001	93.8	0.006	0.011	0.016	0.030	0.132
Pendimethalin	0.067	50.0	<0.067	0.034	0.371	0.968	4.512
Oxadiazines							
Oxadiazon	0.0001	79.3	0.011	0.030	0.046	0.081	0.370
Indoxacarb	0.015	25.8	<0.015	<0.015	0.019	0.449	3.723
Phenylpyrazoles							
Fipronil	0.010	100	0.059	0.143	0.478	17.20	293.9
Fipronil sulfone	0.005	100	0.060	0.130	0.321	2.69	19.78
Carbamates							
Carbaryl	0.038	62.1	<0.038	0.169	0.581	3.316	37.16
Carbendazim	0.286	100	0.596	0.867	1.655	7.571	176.8
Carbofuran	0.006	8.20	<0.006	<0.006	<0.006	0.019	0.130
Fenoxycarb	0.236	0.78	<0.236	<0.236	<0.236	<0.236	2.784
Iprovalicarb	0.033	5.47	<0.033	<0.033	<0.033	0.034	0.635
Methomyl	–	–	–	–	–	–	–
Oxamyl	0.018	8.59	<0.018	<0.018	<0.018	0.029	0.291
Promecarb	–	–	–	–	–	–	–
Propoxur	0.088	69.9	<0.088	0.241	0.522	1.596	14.01
Carboxamidas							
Diflufenican	0.021	75.4	0.024	0.094	0.140	0.318	3.156
Boscalid	0.028	86.7	0.147	0.318	0.691	2.514	67.20
Thiocarbamates							
Prosulfocarb	0.101	100	0.846	1.470	3.100	13.80	29.12
Neonicotinoids							
Acetamiprid	0.004	54.7	<0.004	0.013	0.044	0.148	1.229
Clothianidin	0.019	7.03	<0.019	<0.019	<0.019	0.053	0.330
Dinotefuran	0.016	20.7	<0.016	<0.016	<0.016	0.414	5.823
Imidacloprid	0.024	89.1	0.111	0.305	1.077	21.78	595.7
Thiacloprid	0.007	51.6	<0.007	0.008	0.049	0.334	3.589
Thiamethoxam	0.009	16.8	<0.009	<0.009	<0.009	0.071	1.703
Triazines-Triazones							
Atrazine	0.016	2.34	<0.016	<0.016	<0.016	<0.016	0.129
Atrazine desethyl	0.066	66.4	<0.066	0.230	0.451	0.885	2.043
Prometryn	0.005	20.3	<0.005	<0.005	<0.005	0.100	0.712
Propazine	0.005	32.8	<0.005	<0.005	0.026	0.053	0.271
Sebuthylazine	0.016	14.5	<0.016	<0.016	<0.016	0.275	2.426
Simazine	0.011	10.9	<0.011	<0.011	<0.011	0.028	0.096
Terbuthylazine	0.013	62.9	<0.013	0.049	0.573	1.390	2.248
Terbutryn	0.011	97.3	0.106	0.289	0.770	3.464	39.82
Metamitron	0.368	0.78	<0.368	<0.368	<0.368	<0.368	0.398
Metribuzin	0.184	15.6	<0.184	<0.184	<0.184	1.575	365.1
Chloridazon	0.071	0.78	<0.071	<0.071	<0.071	<0.071	0.406
Urea							
Metoxuron	0.104	28.5	<0.104	<0.104	0.292	4.323	91.32
1-(3,4-dichlorophenyl)-3-methyl-urea	0.009	96.5	0.033	0.054	0.112	0.350	5.176

(*continued on next page*)

Table 2 (*continued*)

Compound	LOD (pg/mg)	Detection frequency (%)	25% Percentile (pg/mg)	50% Percentile (pg/mg)	75% Percentile (pg/mg)	95% Percentile (pg/mg)	Highest value (pg/mg)
1-(3,4-dichlorophenyl)-urea	0.342	100	1.341	2.071	3.725	8.917	40.46
3,4-dichloroaniline	0.747	8.98	<0.747	<0.747	<0.747	2.005	11.23
Chloroxuron	0.377	8.20	<0.377	<0.377	<0.377	0.693	38.46
Chlortoluron	0.090	7.81	<0.090	<0.090	<0.090	0.423	11.53
Diuron	0.049	42.9	<0.049	<0.049	0.225	1.181	26.39
Fenuron	0.007	91.8	0.200	0.622	1.656	5.133	20.98
Isoproturon	0.014	16.8	<0.014	<0.014	<0.014	0.157	17.74
Linuron	–	–	–	–	–	–	–
Methabenzthiazuron	0.010	4.29	<0.010	<0.010	<0.010	<0.010	0.199
Metobromuron	0.013	0.78	<0.013	<0.013	<0.013	<0.013	0.027
Monolinuron	–	–	–	–	–	–	–
Amides pesticides							
Alachlor	–	–	–	–	–	–	–
Dimethalchlor	0.002	31.6	<0.002	<0.002	0.007	0.034	0.084
Fenhexamid	0.429	24.2	<0.429	<0.429	<0.429	17.26	95.93
DMST	0.029	48.4	<0.029	<0.029	0.254	2.841	136.9
Metolachlor	0.005	100	0.024	0.037	0.108	0.214	0.359
Miscellaneous							
Aclonifen	1.131	2.73	<1.131	<1.131	<1.131	<1.131	3.545
Iprodione	0.169	3.52	<0.169	<0.169	<0.169	<0.169	2.229
Spinosyn A	0.001	81.2	0.004	0.009	0.018	0.069	0.407
Crimidine	–	–	–	–	–	–	–
Fenarimol	0.008	1.95	<0.008	<0.008	<0.008	<0.008	0.144
Propargite	0.084	5.08	<0.084	<0.084	<0.084	0.021	0.886
Lenacil	0.088	66.4	<0.088	0.206	0.318	0.740	2.564
Bisphenols							
Bisphenol A	1.930	100	62.34	133.6	338.3	2473.5	35856.1
Bisphenol S	0.273	100	13.39	32.81	84.33	399.4	21632.1

non-persistent chemicals) showed higher concentration in boys, and 9 biomarkers (6 non-persistent pesticides, 2 persistent pesticides and BDE47) showed higher concentration in girls (Table 4, Fig. 1). Influence of sex on exposure was also confirmed by single linear regressions (Table 4), and a multiple linear regression obtaining a $R^2 = 0.34$ (p-value = 6.24E-10).

3.3. Influence of diet on biomarkers' concentration

In order to study the possible influence of diet on exposure, the population under study was divided in two groups based on the information provided in the questionnaire: children following organic diet (n = 61), and children following conventional diet (n = 156) (Table 1). Children following diet composed of ≥ 50% of organic food were included in the group of organic diet. MANOVA test revealed significant differences between the two types of diet (p-value = 0.006). Partial least squares – discriminant analysis (PLS-DA) was used to illustrate the differences between the two groups (Fig. 2). PLS-DA model showed a good measure of separability with an AUC of 0.72 (p-value = 5.12E-07) in the Comp1. Loading values can be found in the table SM1, being boscalid, thiabendazole, azoxystrobin, 3Me4NP, imidacloprid and myclobunatil the variables with the highest loadings (β = 0.28, 0.27, 0.24, 0.23, 0.22 and 0.22 respectively), all positively correlated with conventional diet (table SM1, Fig. 2). Among the 62 biomarkers detected in ≥ 50% of the samples, 17 (16 pesticides and BPS) showed significant differences between the groups, all presenting higher concentrations in children following conventional diet (table SM2, figure SM2). Moreover, all of this variables had high loadings, all positively correlated with conventional diet in the PLS-DA model (table SM1, Fig. 2).

3.4. Influence of the residence area on biomarkers' concentration

Based on the information given by the participants in the questionnaire, children were divided in two groups according to their residence area: urban (n = 123) and countryside (n = 94) (Table 1). MANOVA analysis showed significant differences in exposure between the two groups (p-value = <0.001), and PLS-DA, like in the previous analysis, showed visual differences between the two locations (Fig. 3). PLS-DA model showed a good measure of separability with an AUC of 0.81 (p-value = 9.33E-15) in the Comp1. Loading values can be found in the table SM2, being fipronil, imidacloprid, diflufenican, and fipronil sulfone the variables with the highest loadings (β = 0.29, 0.28, 0.25, and 0.25 respectively), all positively correlated with countryside (table SM1, Fig. 3). Among the 62 biomarkers detected in ≥ 50%, 8 biomarkers showed significant differences between the areas (table SM2). Three biomarkers (PCP, TCPy and prosulfocarb) presented higher concentrations in children living in urban areas, and 5 biomarkers (propiconazole, MCPA, fipronil, diflufenican and imidacloprid) showed higher concentrations in children living in the country side (table SM2, figure SM3). The variables with higher concentration in children living in the countryside showed positive correlations and high loadings in the PLS-DA model, however, the three variables with higher concentration in the urban area showed high negative loadings in the PLS-DA (table SM1, Fig. 3).

3.5. Exposure and presence of pets at home

MANOVA analysis showed significant differences (p-value = <0.001) in exposure between children having pets (n = 86) and children who do not (n = 131) (Table 1). The six biomarkers ($4F_3PBA$, fipronil, fipronil sulfone, imidacloprid, terbutryn and fenuron) that were significantly different between the two groups presented all higher concentrations in children with pets (table SM2, Fig. 4a). Influence of living with pets on exposure was also confirmed with a multiple regression, obtaining a $R^2 = 0.46$ (p-value < 0.001).

MANOVA analysis showed significant differences (p-value = <0.001) between children living in households where pets are treated with anti-parasites (n = 47) and children whose pets are not treated (n = 39) (Table 1). Nine biomarkers showed significant differences between these two groups. Five biomarkers (PNP, fipronil, fipronil sulfone, imidacloprid and lenacil) where detected at higher concentrations in the group that uses anti-parasites (table SM2, Fig. 4b), and four biomarkers (metolachlor, DMTP, terbuthylazine and fenuron) were detected at higher concentrations in the group that does not use anti-parasites. The effects of anti-parasites use on exposure was confirmed as well by a multiple regression, obtaining a $R^2 = 0.46$ (p-value < 0.001).

Table 3
Correlation and single regression models between biomarkers' concentration and children's age1.

Compound	Pearson Correlation		Spearman correlation		Linear single regression between exposure and age					
	Coefficient	*p-value*	Coefficient	*p-value*	β	R^2	*p-value*	Confidence Interval (2.5% − 97.5%)	Percent of change β	Percent of change (CI 2.5% − 97.5%)
αEndosulfan	−0.12	0.06	−0.09	0.12	−0.04	0.04	0.04*	−0.08 - −0.002	−4.04	−7.69 - −0.23
DEP	−0.22	<0.001*	−0.22	<0.001*	−0.02	0.08	<0.001*	−0.03 - −0.01	−2.24	−3.48 - −0.98
P-Nitrophenol (PNP)	−0.27	<0.001*	−0.26	<0.001*	−0.02	0.09	1.21E-05*	−0.02 - −0.01	−1.52	−2.19 - −0.85
3Me4NP	−0.29	<0.001*	−0.27	<0.001*	−0.03	0.15	1.84E-06*	−0.04 - −0.02	−2.56	−3.57 - −1.53
Malathion	−0.44	<0.001*	−0.47	<0.001*	−0.16	0.23	8.99E-14*	−0.19 - −0.12	−14.5	−17.8 - −11.1
$ClCF_3Ca$	−0.14	0.03*	−0.03	0.63	−0.04	0.03	0.02*	−0.07 - −0.01	−3.82	−6.97 - −0.57
4F3PBA	−0.22	<0.001*	−0.21	<0.001*	−0.05	0.04	<0.001*	−0.07 - −0.02	−4.39	−6.78 - −1.95
Difenoconazole	−0.42	<0.001*	−0.40	<0.001*	−0.09	0.17	4.75E-12*	−0.11 - −0.07	−8.61	−10.8 - −6.35
Imazalil	−0.16	0.01*	−0.16	0.01*	−0.05	0.02	0.01*	−0.09 - −0.01	−4.84	−8.35 - −1.19
Propiconazole	−0.31	<0.001*	−0.24	<0.001*	−0.06	0.09	9.28E-07*	−0.09 - −0.04	−6.13	−8.43 - −3.78
Tebuconazole	−0.28	<0.001*	−0.29	<0.001*	−0.04	0.11	9.03E-06*	−0.07 - −0.03	−4.54	−6.44 - −2.59
Thiabendazole	−0.35	<0.001*	−0.36	<0.001*	−0.06	0.13	9.30E-09*	−0.08 - −0.04	−6.06	−7.98 - −4.09
Azoxystrobin	−0.38	<0.001*	−0.38	<0.001*	−0.08	0.14	2.95E-10*	−0.11 - −0.06	−7.89	−10.1 - −5.59
Pyraclostrobin	−0.28	<0.001*	−0.28	<0.001*	−0.06	0.07	4.18E-06*	−0.08 - −0.03	−5.37	−7.53 - −3.16
Trifloxystrobin	−0.34	<0.001*	−0.29	<0.001*	−0.07	0.11	2.32E-08*	−0.09 - −0.05	−7.06	−9.36 - −4.71
Mecroprop	−0.21	<0.001*	−0.23	<0.001*	−0.02	0.06	<0.001*	−0.04 - −0.01	−2.30	−3.63 - −0.96
2,4-D	−0.26	<0.001*	−0.29	<0.001*	−0.04	0.08	2.72E-05*	−0.06 - −0.02	−3.95	−5.72 - −2.15
2,4-DB	−0.35	<0.001*	−0.39	<0.001*	−0.14	0.12	9.18E-09*	−0.18 - −0.09	−12.8	−16.7 - −8.76
Pyrimethanil	−0.39	<0.001*	−0.35	<0.001*	−0.13	0.16	1.47E-10*	−0.17 - −0.09	−11.9	−15.2 - −8.76
Pendimethalin	−0.17	0.005*	−0.17	0.008*	−0.04	0.03	0.005*	−0.07 - −0.01	−3.93	−6.54 - −1.25
Oxadiazon	−0.23	<0.001*	−0.19	0.001*	−0.08	0.06	<0.001*	−0.13 - −0.04	−8.02	−12.1 - −3.77
Carbaryl	−0.49	<0.001*	−0.48	<0.001*	−0.15	0.25	2.86E-17*	−0.18 - −0.11	−13.6	−16.2 - −10.8
Carbendazim	−0.24	<0.001*	−0.33	<0.001*	−0.03	0.17	9.83E-05*	−0.04 - −0.01	−2.65	−3.65 - −1.34
Propoxur	−0.36	<0.001*	−0.34	<0.001*	−0.06	0.12	7.15E-09*	−0.08 - −0.04	−6.07	−7.99 - −4.12
Diflufenican	−0.19	0.003*	−0.13	0.03*	−0.03	0.03	0.003*	−0.05 - −0.01	−2.87	−4.70 - −1.01
Boscalid	−0.41	<0.001*	−0.38	<0.001*	−0.08	0.18	1.54E-11	−0.10 - −0.06	−7.67	−9.69 - −5.59
Prosulfocarb	−0.22	<0.001*	−0.19	0.002*	−0.03	0.04	<0.001*	−0.05 - −0.01	−3.03	−4.68 - −1.36
Acetamiprid	−0.39	<0.001*	−0.40	<0.001*	−0.12	0.15	1.04E-10*	−0.15 - −0.08	−10.9	−13.9 - −7.87
Imidacloprid	−0.25	<0.001*	−0.27	<0.001*	−0.07	0.09	5.02E-05*	−0.10 - −0.04	−6.66	−9.68 - −3.54
Thilacloprid	−0.29	<0.001*	−0.31	<0.001*	−0.08	0.09	3.35E-06*	−0.12 - −0.05	−7.99	−11.1 - −4.76
Atrazine desthyl	−0.44	<0.001*	−0.46	<0.001*	−0.07	0.19	2.27E-13*	−0.07 - −0.06	−7.21	−8.95 - −5.43
Terbuthylazine	−0.17	0.006*	−0.14	0.03*	−0.06	0.03	0.01*	−0.06 - −0.02	−5.57	−9.43 - −1.56
Terbutryn	−0.19	0.003*	−0.26	<0.001*	−0.04	0.03	0.003*	−0.03 - −0.01	−3.44	−5.66 - −1.18
Fenuron	−0.35	<0.001*	−0.31	<0.001*	−0.08	0.16	1.21E-08*	−0.08 - −0.05	−7.89	−10.4 - −5.33
Spinosyn A	−0.26	<0.001*	−0.22	<0.001*	−0.06	0.09	1.19E-05*	−0.06 - −0.03	−5.59	−7.96 - −3.17
Lenacil	0.14	0.03*	0.15	0.02*	0.01	0.02	0.03*	−0.02 - −0.03	−1.80	0.14 - −3.49
Bisphenol A	−0.17	0.005*	−0.19	0.002*	−0.03	0.08	0.01*	−0.05 - −0.01	−2.85	−4.89 - −0.76
Bisphenol S	−0.45	<0.001*	−0.44	<0.001*	−0.08	0.24	2.59E-14*	−0.09 - −0.06	−7.67	−9.45 - −5.86

[1]Biomarkers with detection frequency ≥ 50% that showed significant correlation with age.
*Significant result when p-value < 0.05.

4. Discussion

The present study demonstrates the exposure of Luxembourgish children to multiple compounds from different families simultaneously. Indeed, the number of biomarkers detected per child ranged from 29 to 88 (61 on average), and only 17 biomarkers out of the 153 compounds tested here were not detected in any of the samples (Table 2). These results are in line with previous studies conducted in other regions on smaller groups of children, but also demonstrating multiple exposure (Iglesias-González et al. 2020; Iglesias-González et al. 2021; Palazzi et al. 2019). Although the impact of cumulative exposure on health is increasingly pointed out (Borman et al. 2017; Silva et al. 2002; Song et al. 2016), the assessment of exposure to multiple pollutants and the associated risks remains highly challenging (Hernández et al. 2017; Iglesias-González et al. 2021). Yet, most studies still resort on single compound, therefore providing information that is not representative of reality and cannot be used by regulatory agencies to establish "safe" exposure levels (Hernández et al. 2017; Kostoff et al. 2018). Surprisingly, children presented high detection frequencies for many persistent pesticides, PCBs and BDEs, many of them being detected in ≥ 50% of the samples (Table 2). Although the presence of POPs in adults is often attributed to past exposure and lifelong bioaccumulation (Geyer et al. 2000), this hypothesis falls short for children due to their young age. The presence of POPs in children's hair therefore strongly suggests that exposure is still ongoing, even though these compounds have been banned in Europe for more than 20 years (Stockholm Convention 2018). Industry and waste of obsolete industrial areas are the major source of POPs in the environment (Jacob and Cherian 2013); in consequence, the strong industrial past of Luxembourg, in addition to the slow degradation of these chemicals could explain children exposure to POPs. In parallel exposure to POPs due to the consumption of contaminated imported food cannot be excluded.

The associations of biomarkers concentration with age and sex are in line with previous studies conducted on children exposure (Iglesias-González et al. 2020; Iglesias-González et al. 2021; Palazzi et al. 2019). Indeed, age was associated with 37 biomarkers out of 59 (DF ≥ 50%), showing significant negative correlations between age and concentration, indicating systematically higher exposure in younger children for all biomarkers (Table 3). Although the specific behaviour of young children (e.g. hand-to-mouth, playing on the floor, breastfeeding) or age-related food consumption could lead to higher exposure, especially in toddlers (Makri et al. 2004), metabolic differences might also explain the inverse association between age and exposure suggested here. Indeed, faster metabolism, respiratory rate and faster intestinal absorption, which are inversely associated with age, could lead to higher exposure in younger children (Griffiths et al. 1990; Makri et al. 2004; Molnar and Schutz 1997). Sex was significantly associated with 28 biomarkers, which showed significant differences between boys and

Table 4
Differences between biomarkers' concentration regarding children's sex[1] and single regression models sowing associations between exposure and sex.

Compound	Females			Males			*p-value*	Linear single regression between exposure and sex					
	25th	50th	75th	25th	50th	75th		β Sex[2]	R^2	*p-value*	Confidence Interval (2.5% – 97.5%)	Percent of change β	Percent of change (CI 2.5% – 97.5%)
y-HCH	0.13	0.33	0.79	0.12	0.23	0.46	0.03*	−0.19	0.03	0.01*	−0.36 - −0.04	−18.1	−29.9 - −4.33
α-Endosulfan	0.001	0.02	0.04	0.000	0.01	0.03	0.005*	−0.41	0.04	0.004*	−0.68 - −0.13	–33.5	−49.5 - −0.23
IMPy	0.002	0.01	0.05	0.003	0.04	0.08	0.01*	0.27	0.02	0.02*	0.05–0.48	30.3	4.75–1.96
DEP	0.67	1.04	1.91	0.92	1.69	2.66	<0.001*	0.16	0.09	<0.001*	0.07 – 0.25	17.5	7.36 - −0.99
TCPy	0.25	0.49	1.07	0.19	0.32	0.60	<0.001*	−0.18	0.04	<0.001*	−0.27 - −0.08	−16.3	–23.9–1.17
P-Nitrophenol (PNP)	5.44	7.79	10.1	5.96	9.24	11.9	0.01*	0.06	0.09	0.02*	0.01 – 0.11	6.14	1.18 - −0.85
3Me4NP	0.25	0.47	0.74	0.44	0.70	1.05	<0.001*	0.17	0.15	9.99E-6*	0.09 – 0.24	18.4	9.99 - −1.53
Malathion	0.01	0.07	1.02	0.03	0.91	2.47	<0.001*	0.54	0.23	<0.001*	0.26 – 0.81	70.8	29.4 - −11.1
Permethrine	10.0	22.5	49.4	4.12	11.5	47.5	0.005*	−0.22	0.01	0.02*	−0.41 - −0.04	−19.8	–33.4 – 3.34
$ClCF_3Ca$	0.02	0.07	0.16	0.01	0.05	0.11	0.01*	−0.29	0.03	0.02*	−0.52 - −0.05	−24.9	−40.7 - −0.57
3-PBA	0.26	0.39	0.96	0.37	0.61	1.38	<0.001*	0.19	0.04	<0.001*	0.08 – 0.29	20.5	8.16 - −0.56
BDE 47	0.04	0.22	0.46	0.03	0.08	0.34	0.02*	−0.23	0.02	0.01*	−0.40 - −0.06	−20.6	–33.2 – 3.93
Tebuconazole	0.16	0.29	0.49	0.18	0.30	0.64	0.09	0.23	0.11	0.001*	−0.07 - −0.03	26.4	9.59 - −2.59
Thiabendazole	0.45	1.06	4.23	0.76	1.80	4.09	0.04*	0.13	0.13	0.08	−0.02 – 0.27	13.7	−1.79 - −4.09
Mecroprop	0.19	0.40	0.71	0.28	0.47	0.77	0.04*	0.13	0.06	0.01*	0.03 – 0.23	13.8	3.30 - −0.96
MCPA	0.14	0.37	1.11	0.20	0.48	1.42	0.02*	0.15	0.02	0.02*	0.02 – 0.27	16.2	2.53 – 3.44
2,4-D	0.13	0.19	0.36	0.16	0.25	0.54	0.01*	0.15	0.08	0.02*	0.02 – 0.28	16.3	1.98 - −2.15
Trifluralin	0.01	0.01	0.02	0.01	0.01	0.01	0.001*	−0.13	0.02	0.02*	−0.25 - −0.02	−12.6	−21.9 – 2.69
Oxadiazon	0.000	0.02	0.04	0.02	0.03	0.05	0.04*	0.34	0.06	0.04*	0.02 – 0.66	39.8	1.52 - −3.77
Fipronil sulfone	0.08	0.17	0.42	0.05	0.09	0.28	0.01*	−0.18	0.02	0.02*	−0.34 - −0.03	−16.8	−28.5 – 2.61
Carbendazim	0.51	0.67	0.93	0.74	1.13	2.37	<0.001*	0.29	0.17	4.91E-9*	0.19 – 0.39	33.8	21.7 - −1.34
Boscalid	0.06	0.27	0.66	0.19	0.37	0.69	0.04*	0.18	0.18	0.02*	0.03 – 0.34	20.2	2.74 - −5.59
Imidacloprid	0.07	0.24	0.71	0.14	0.40	1.65	0.004*	0.37	0.09	0.002*	0.14 – 0.60	44.6	14.6 - −3.54
1-(3,4-dichlorophenyl)-urea	1.21	1.77	3.04	1.49	2.31	4.52	0.003*	0.13	0.04	0.003*	0.05 – 0.21	13.8	4.65 – 0.26
Fenuron	0.14	0.31	1.12	0.37	0.85	1.88	<0.001*	0.37	0.16	<0.001*	0.17 – 0.56	44.7	19.1 - −5.33
Spinosyn A	0.01	0.01	0.02	0.003	0.01	0.02	<0.001*	−0.25	0.09	0.01*	−0.43 - −0.07	–22.3	−35.0 - −3.17
Bisphenol A	46.1	92.3	235.2	88.2	162.7	470.3	<0.001*	0.31	0.08	7.31E-5*	0.16 – 0.46	36.1	17.1 - −0.76
Bisphenol S	9.28	24.7	68.4	19.3	42.9	110.4	<0.001*	0.28	0.24	8.68E-5*	0.14 – 0.42	32.1	15.1 - −5.86

[1]Biomarkers with detection frequency ≥ 50% and that showed significant differences between sexes
[2]In the regression model, 1 was given to the females and 2 to the males
*Significant result when p-value < 0.05
Concentrations are presented in pg/mg

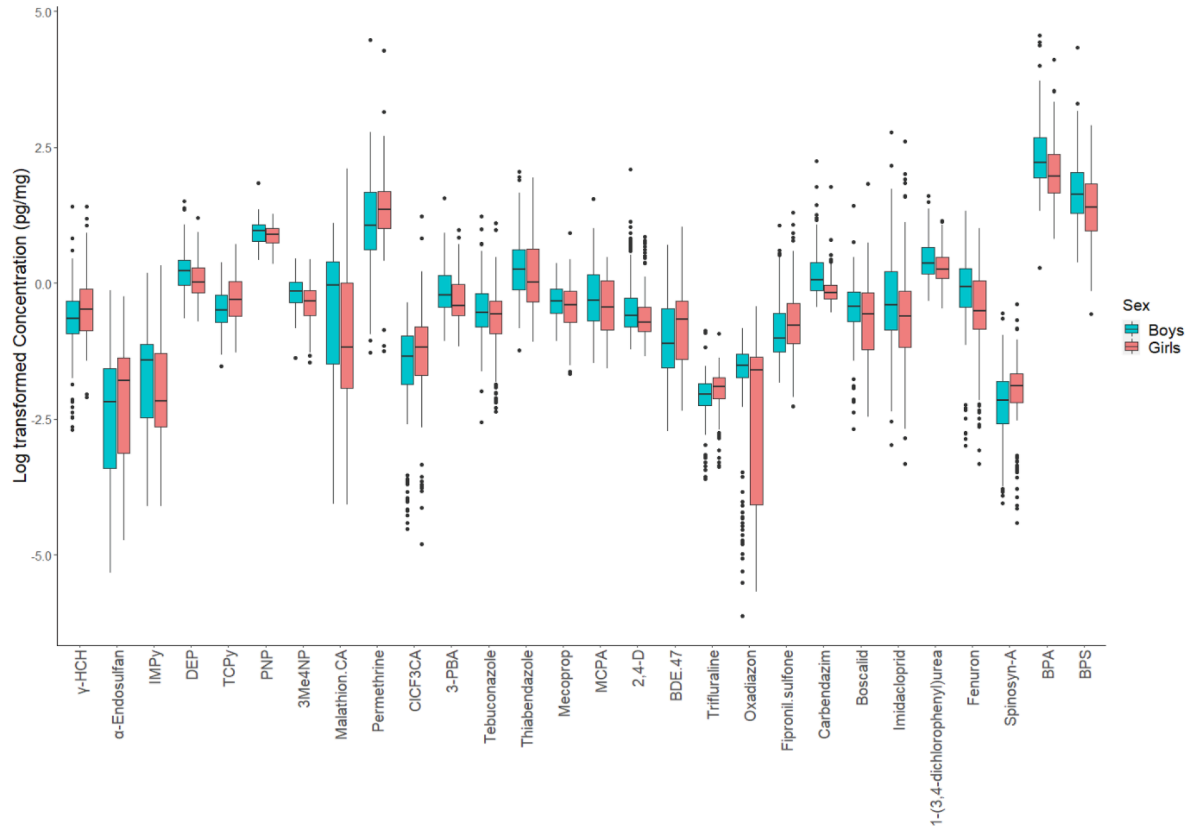

Fig. 1. Boxplot representation of the concentration in boys and girls of biomarkers that showed significant differences in concentration between sexes. Eighteen biomarkers showed higher concentration in boys and nine biomarkers showed higher concentration in girls.

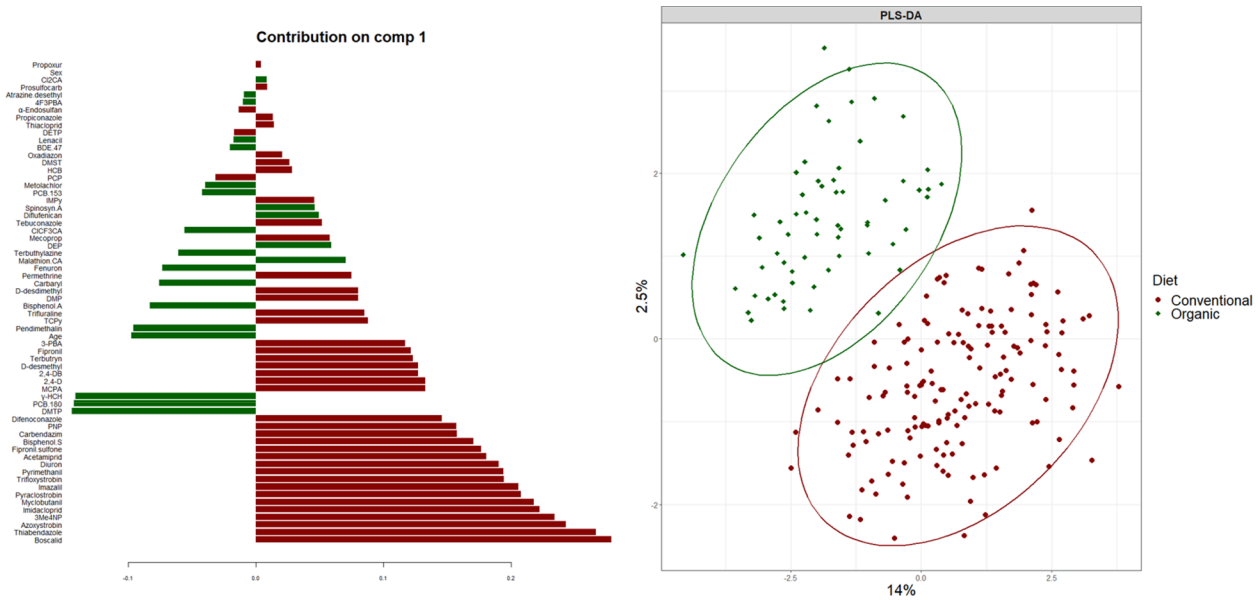

Fig. 2. Graphic representation of the PLS discriminant analysis comparing the exposure of children following organic and conventional diet. On the left, contribution of each biomarker on the exposure by each type of diet, and on the right, representation of each child by dot and each diet by colour, showing a clear separation between children that follow organic diet from the ones following conventional diet.

girls: 19 biomarkers (all non-persistent chemicals) showed higher concentrations in boys and 9 biomarkers in girls (6 non-persistent pesticides, 2 persistent pesticides and BDE47) (Fig. 1, Table 4). In adults, the gender differences observed regarding pollutant concentration in biological matrices are usually attributed to metabolism, body composition and daily habits, pointing out that women are usually more exposed than men due to their diet preferences and body fat content (Garcia 2003; Qiao et al. 2016; Stubleski et al. 2018). Although these statements cannot be strictly applied to children because sexual dimorphism regarding body composition is less marked than in adults (Wells et al. 2007), pre-pubertal children also present physiological and behaviour dimorphism between sexes. For instance, it has been demonstrated that

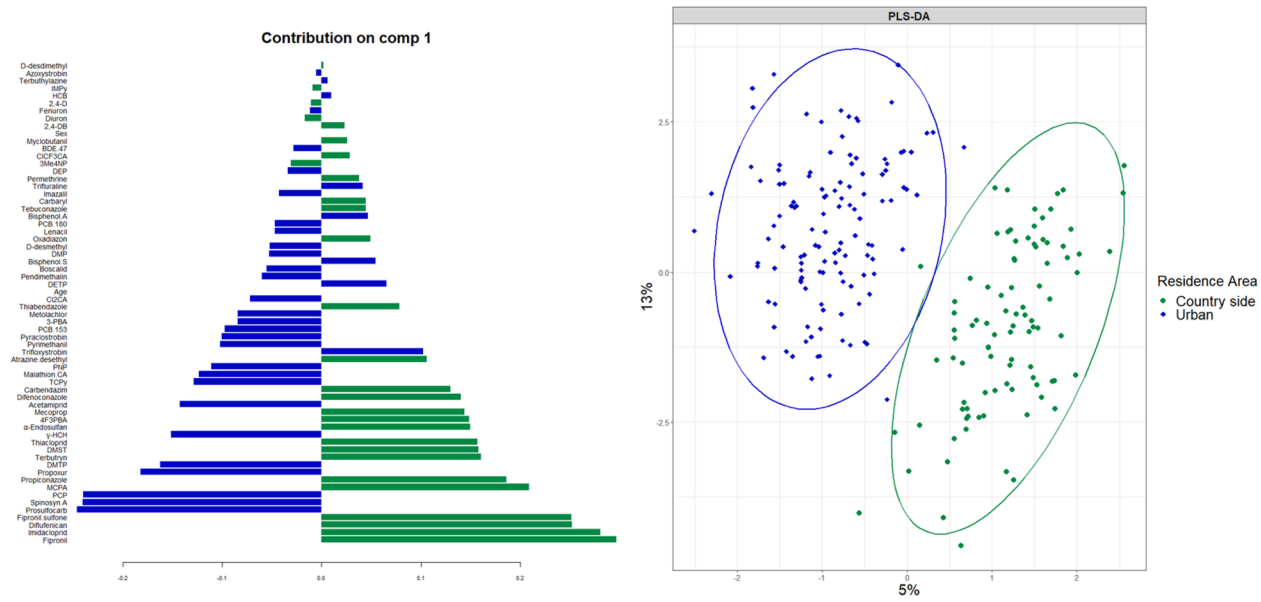

Fig. 3. Graphic representation of the PLS discriminant analysis comparing the exposure of children living in urban our countryside residence areas. On the left, contribution of each biomarker the exposure by each residence area, and on the right, representation of each child by dot and each area by colour, showing a clear separation between children that live in urban areas from the ones living in the countryside.

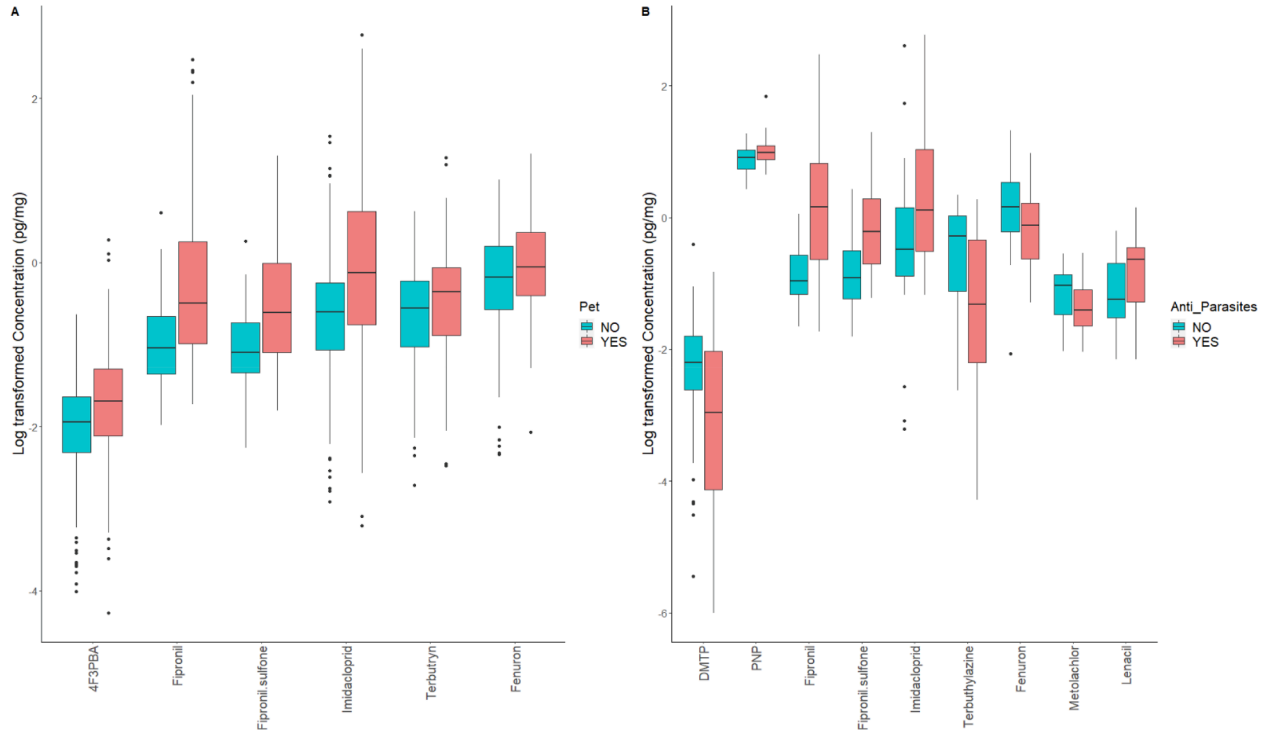

Fig. 4. A) Boxplot representation of the biomarkers that showed significant differences in concentration between children who live with pets and children who do not. B) Boxplot representation of the biomarkers that showed significant differences in concentration between children who live with pets treated with anti-parasites and children who do not.

boys have less body fat than girls, even though the difference is milder than in adults (Taylor et al. 1997). The three POPs that presented significantly different concentration between sexes (γ-HCH, α-endosulfan and BDE47) showed higher concentration in girls, which would be in line with the hypothesis of an association with the amount of body fat in the body (Barrett 2013). In counterpart, most of the non-persistent pollutants presenting significant differences between sexes showed higher concentration in boys, rather suggesting a link with sex-specific behaviour. In children, higher exposure in males could therefore be associated with gender-specific activities possibly increasing exposure (e.g. football paying on contaminated fields) and the amount of food they consume, which are both usually higher than in girls for the same age and weight (Bellisle and Rolland-Cachera 2007; Molnar and Schutz 1997).

Diet is generally considered one of the main contributors of exposure to most organic pollutants, particularly pesticides (EFSA et al. 2021).

The European coordinated control program analysed in 2019 more than 12,000 samples of conventional food, from which 45% contained one or more pesticide residues below or equal to the Maximum Residue Levels (MRL), and 2% of the samples showed pesticide residues exceeding the MRL (EFSA et al. 2021). Even though the increasing awareness of the adverse effects of pesticides on health and on the environment stimulated efforts to decrease their use for food production, conventional agriculture remains largely predominant and organic agriculture only increased from 5.7 to 8.5% over the period of 2012–2019 in EU (Eurostat 2021). Similar tendency was also observed in food preference, and the perception of the health benefit through exposure reduction was among the main drivers towards organic food consumption (Brantsæter et al. 2017). In the present study, the 17 biomarkers that were significantly different between children under organic versus conventional diet presented all higher concentrations in the hair samples of the children following conventional diet (figure SM2, table SM2). These results are in line with several publications endorsing that organic food consumption could lead to a reduction of the exposure to pesticides through diet. For instance, Curl et al. (2003) reported significantly lower concentration of urinary metabolites of organophosphates in children consuming organic food compared to children consuming conventional diet. Similarly, Lu et al. (2006) and Hyland et al. (2019) demonstrated significant reduction in urinary pesticide metabolites in children and adults, following a switch from conventional diet to organic food. From the 17 biomarkers that showed higher concentration in children who follow conventional diet, only diuron is completely banned in Europe, and imidacloprid, fipronil and acetamiprid are banned from being used outdoors due to the harm they do to pollinator insects (European Commission, 2021; PAN, 2018). Despite the efforts made at the national and EU level to reduce pesticide use, many compounds including diuron, imidacloprid and acetamiprid, were detected in the water of the Luxembourgish rivers (Krier et al. 2022). This suggests current use for agriculture purpose, which could explain exposure through consumption of locally produced conventional food. On top of that, a significant part of the food consumed in the country is imported, principally eggs, vegetables, chicken and fruits, of which Luxembourg only generates 35%, 3–5%, 1.4% and < 1% respectively of the total demand (Best Food Importers, 2020). Food consumables are mainly imported from EU, especially from Germany, France and Belgium, and approximately 10% comes from outside EU, principally from Asia and United States (Best Food Importers, 2020; European Commission, 2016; The World Bank, 2018). Differences in local legislation on pesticide use might therefore also explain the exposure to chemicals not used in Luxembourg (Claeys et al. 2011; Nougadère et al. 2012; Thompson and Darwish 2019).

Contrary to diet, the differences in exposure associated with residence area were not unidirectional since 3 compounds presented higher concentration for children living in urban areas whereas 5 were higher in children living in rural areas (figure SM3, table SM2). The biomarkers that showed higher concentrations in urban children were PCP, TCPy and prosulfocarb. Although PCP is already banned in Europe, it was used, among other functions, as a component in antifouling paint for wood preservation until few decades ago (Stockholm Convention 2018); hence, it could still be present in Luxembourgish homes. Prosulfocarb and chlorpyrifos (the parent pesticide of TCPy) are worldwide used pesticides in crops and fruits respectively. Differences in exposure might therefore be due to the origin of the imported food, which may be different between rural and urban areas. The five pesticides showing higher concentrations in children living in rural areas, were all still allowed in Europe but one, diflufenican, for which the European Union did not renew the license for use in 2019 (ECHA 2021). Although agriculture only represents 0.3% of the Luxembourgish economy, green areas cover the Country almost entirely (European Commission 2016), and according to The World Bank (2018) 60% of Luxembourgish land is dedicated to agriculture (37.7%) and to milk production (61.4%). From the total land assigned to agriculture, only 5% is currently designated to organic production (Best Food Importers, 2020; PAN Bio 2020). Environmental contamination associated with pesticide use and subsequent exposure might therefore concern a large part of the population living close to agriculture areas (crops, vineyards, orchards…). In parallel, the special distribution of Luxembourgish land makes it difficult to clearly differentiate urban areas and countryside, and might have hidden some differences in exposure between urban and rural areas. Actually, 78% of Luxembourgish living areas are considered rural, and although 70% of the population live in urban areas, the two biggest cities only represent 51.5 km2 and 14.3 km2, and are directly surrounded by agricultural areas (European Commission 2016). Finally, as observed for imidacloprid, significant differences in exposure were also observed for other parameters than location (Table SM2). The multiple sources of exposure could also mask the contribution of each, explaining overlaps and even hide some differences. The role of environmental contamination due to agriculture in exposure to pesticides cannot be excluded, and additional research with a specific design would be necessary to better understand the impact of pesticide use on local contamination and human exposure. In addition to the previous variables, the presence of pets at home was also identified as a determinant of exposure to some pesticides. Indeed, previous studies demonstrated that anti-parasites applied to pets were rapidly transferred to pet owner, especially children who generally have closer contact with the animals and with materials contaminated post application (Cochran et al. 2015). In the present study, 6 compounds showed significantly higher concentrations in children with pets (table SM2) and, more specifically, five biomarkers showed higher concentrations in children who use products against parasites (table SM2). Precisely, imidacloprid and fipronil were both detected at significantly higher concentrations in children living with pets treated with anti-parasites (1.29 vs 0.33 pg/mg and 1.45 vs 0.11 pg/mg respectively). The 2 latter pesticides are the active agents of six different brands of commercial anti-parasites that were disclosed by the families in the questionnaires (Fig. 4, table SM2). Fipronil and imidacloprid have been associated with several adverse health effects in humans. Fipronil is reported to not come off the skin once it is dry, which increases the dermal contact (Tingle et al. 2000). Short-term exposure to commercial products containing fipronil has been associated with ocular, skin, and respiratory reactions. Long-term exposure to fipronil has been associated with endocrine disruption, and in specific, fipronil and fipronil sulfone have showed “anti-thyroid hormone activities”, affecting the normal regulation of estrogens (Lu et al. 2015; Tingle et al. 2000). Moreover, fipronil has been classified as carcinogenic by the US-EPA (Tingle et al. 2000). In parallel, in vitro studies conducted on breast cancer cells demonstrated that environmental concentration of imidacloprid might induce changes in CYP19 promoter usage, similar to that observed in hormone-dependent breast cancer patients (Caron-Beaudoin et al. 2018).

5. Strengths and limitations of the study

The present study is the first one investigating such a great number of biomarkers from different chemical classes, and the possible determinants of exposure in such an extensive number of children. The use of hair for exposure assessment can only provide information on chronic exposure, but is not adapted to the biomonitoring of short-term exposure, which better appreciated by means of fluids analysis. Nevertheless, in the context of the present study, information on chronic exposure seems more suitable to identify determinants such as diet, residence area or presence of pets at home. On top of that, information regarding the exposure to such an extensive number of biomarkers, including parents and metabolites, can only be obtained with hair analysis, and not with fluids, e.g. urine and blood, where only metabolites or parents can be detected, respectively. However, the limited quantity of hair available (usually around 40–100 mg, compared to several mL for fluids), and the complexity of extracting chemicals from a solid matrix compared to fluids, requires more sophisticated pre-analytical treatment.

Since no specific criteria regarding socio-economical aspects were

applied in this study, this sample cannot be considered representative of the general population, and the over-representation of some categories (e.g. higher socio-economical levels) cannot be excluded. Similarly, the number of children was not homogenous among the different age categories, with a higher proportion in the range 1–8 years old. Additionally, obtaining the volunteers information by self-reported questionnaires could be considered a bias. Nevertheless, none of these aspects was suspected to create "artificial associations" between exposure and information collected in the questionnaires.

The results and interpretation presented in this study are specific both to the chemicals analysed and to the population under study. As a result, different associations could be identified in similar studies conducted with different analytical methods or in regions with different food supply chain, agricultural practice, or urban environment. The global conclusions would however probably remain valid, although pointing out different chemicals.

6. Conclusion

The present study strongly confirms that hair analysis is particularly well adapted to investigate children exposure to environmental pollutants. The extensive number of pollutants detected demonstrates that, alike adults, children are subjected to simultaneous exposure to multiple pollutants from different chemical families.

The results also demonstrate that children, and not only adults, are exposed to many persistent organic pollutants (e.g. pesticides, PCBs and BDEs). Moreover, due to the young age of the participants, the presence of POPs in their hair cannot be attributed to bioaccumulation associated with past exposure. This therefore strongly suggests that, although these compounds have been banned for more than two decades, exposure is still currently ongoing.

Demonstrating that children exposure is associated with both diet and residence area, the present work also provides insight into the identification of the determinants of exposure and suggests possibilities for interventions aiming at reducing it.

The results obtained here set the basis for further investigations following more specific design, to better understand the contribution of the different sources of exposure to pollutants in children.

CRediT authorship contribution statement

Alba Iglesias-González: Conceptualization, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Mylène Schweitzer:** Formal analysis. **Paul Palazzi:** Methodology, Validation. **Fengjiao Peng:** Software, Formal analysis. **Serge Haan:** Writing – review & editing, Project administration, Funding acquisition. **Elisabeth Letellier:** Writing – review & editing, Project administration, Funding acquisition. **Brice M.R. Appenzeller:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Visualization, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107342.

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