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**Abstract**

**Background:** The Danish part of the large European Human biomonitoring pilot project Demonstration of a study to Coordinate and Perform Human biomonitoring on a European Scale (DEMOCOPHES) investigated the urine, hair and blood concentrations of 66 different environmental chemicals in a group of 145 Danish school children aged 6–11 years and their mothers from rural and urban areas in autumn 2011. Some – but not all – results were published; however, the concurrence of the chemicals has not been assessed.

**Methods:** The measured concentrations of polybrominated diphenyl ethers (PBDEs) and glyphosate is assessed to complete the investigation of all 66 chemicals in DEMOCOPHES. The concentrations of PBDEs were measured in plasma samples of 143 mothers and 116 children. Glyphosate was measured in a subsample of 27 urine samples. Previously assessed chemicals were polychlorinated biphenyls (PCBs), and polyfluoroalkyl substances (PFASs) analyzed in blood samples, mercury analyzed in hair, and phthalate metabolites, parabens, phenols, cadmium, paracetamol and cotinine analyzed in urine samples. Differences in concentrations between mothers and children were assessed, and the associations between the concentrations of the different environmental chemicals. investigated by correlation analysis.

**Results:** PBDE47 was found in relatively high levels compared with previous Danish results in both mothers and children, with a significantly higher level in the children compared to their mothers. Glyphosate in concentrations around 1 ng/mL was detected in all 27 samples. The analyzed environmental exposures seem to follow a pattern where chemicals within the same classes are strongly correlated and where children and mothers are exposed to the same chemicals.

**Conclusion:** The correlations between the measured environmental chemicals indicate that a specific exposure pattern may exist, where people who are highly exposed to one class of environmental chemicals also may be highly exposed to certain other classes. As some of the compounds were measured in higher levels in children compared to mothers, increased focus also on the exposure in young children is recommended. For more detailed investigation of specific exposure sources more studies with increased power and detailed questionnaires should be developed.

**Keywords:** children; DEMOCOPHES; exposure patterns; glyphosate; PBDE.

## Introduction

A large variety of chemicals are added to consumer products with the purpose to ease and simplify various daily activities such as cooking, protection and cleaning of clothes and fabric as well as prevention of fires in electronics and furniture. Many of these chemicals are potentially harmful to humans if absorbed. Unfortunately, in many cases these chemicals do not stay in the product of intention, but gradually leach from the product, thereby exposing the consumers. New chemicals are continuously produced and some old chemicals such as persistent organic pollutants (POPs) are not rapidly degraded and can stay in the environment even long after their

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production and use in consumer products have been banned. Moreover, these chemicals may accumulate in the food chain which leads to increased human exposure. Human absorption of these chemicals can occur through oral ingestion, inhalation and dermal absorption.

An important tool in the investigation of chemical exposure in humans is human biomonitoring (HBM) where the concentration of a chemical, its metabolites or an early biomarker of effect is measured in a suitable sample of either the general population or people exposed to high levels of the chemical; either due to an accident, occupational exposure or similar. Particularly, HBM is useful in the assessment of time- or location-specific exposure-trends, and in identifying and investigating exposure in vulnerable groups (1).

A HBM framework was established by the Consortium to Perform Human biomonitoring on European Scale (COPHES), and a pilot study Demonstration of a study to Coordinate and Perform Human biomonitoring on a European Scale (DEMOCOPHES) was set up to test the feasibility of a harmonized HBM framework in Europe with the possibilities to compare exposure levels across borders and support environmental, health and chemical policies. In total 17 countries participated and sampling was performed from September 2011 to February 2012 (2). DEMOCOPHES gathered information on children aged 6–11 years and their mothers through questionnaires on lifestyle and diet, and hair and urine samples. Results from the full European study have been published by Den Hond et al. (2) and in a special edition of *Environmental Research* (3).

The Ministries of Health, Environment and Food Safety in Denmark requested and financed an expansion of the study by adding blood sampling of the participants to the Danish part of DEMOCOPHES. These blood samples were analyzed for content of several POPs, micronuclei and dioxin-like activity. The study of Danish urine samples was also expanded and analyzed for more than the standard DEMOCOPHES set of analyses.

Adverse effects associated with exposure to individual chemicals have been studied widely. However, the population is exposed to a mixture of many chemicals simultaneously and the health effects might therefore be a result of the total exposure to multiple chemicals. The health effects of mixtures of chemicals may not be the sum of the individual effects, which has been shown in animal studies (4, 5).

In the present study, we assess the mixed exposure to a set of chemicals of many different origins and include both non-persistent and persistent chemicals; both from the standard DEMOCOPHES analyses and the additionally obtained measurements. The persistent chemicals are measured in the blood and reflect exposure through months or years and are affected by bioaccumulation of the chemicals. These include biomarkers of polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), dichlorodiphenyltrichloro-ethane (DDT), polyfluoroalkyl substances (PFASs) and polybrominated diphenyl ethers (PBDEs) which are all classified as (POPs). The non-persistent biomarkers were measured in the urine and reflect an exposure within the last 24 h due to their short half-lives (6). These include phthalate metabolites, parabens, phenols and the analgesic paracetamol in the full study population and the pesticide glyphosate in a subgroup. In Denmark, and six other countries the study was expanded by including bisphenol A (BPA) measurements in the urine (7). The individual exposures to many of these chemicals have been reported previously (8–14), but not the exposures to PBDEs – used as flame retardants – and glyphosate –a widely used herbicide.

PBDEs are synthetically produced compounds used as additives to retard fire and flames in a variety of commercial and household products such as furniture and electronics. Structurally PBDEs are similar to PCBs hence the nomenclature is based in the system developed for the PCBs. They are classified as POPs, that are highly bioaccumulative and biomagnify in fat tissues in fish and mammals including humans. Humans are primarily exposed through inhalation of dust, dermal absorption and intake of fish, poultry, meat and dairy products. PBDEs are toxic and studies in animals and epidemiological or HBM studies in humans have shown neurotoxic, endocrine disrupting and carcinogenic effects (15–18).

A subset of the Danish urine samples was also analyzed for glyphosate content. Glyphosate is the most widely used herbicide in the world, and has been in use since 1974 (19). In 1996, the first genetically engineered crops resistant to glyphosate were planted for commercial use in the USA, and the use of glyphosate-products has since risen markedly (20). The available studies of presence of glyphosate in human urine suggest that exposure differs between countries, and that even consumers who buy predominantly organic products are exposed. The general public is mostly exposed to glyphosate through diet or use of glyphosate products in private gardens.

In the present paper, we summarize the exposure to the previous reported biomarkers (8–14), and report data on exposure to PBDE and glyphosate. Furthermore, we assess the correlation between the exposures to the persistent environmental chemicals.

# Materials and methods

## Recruitment

The recruitment of participants and collection of samples followed the COPHES/DEMOCOPHES developed protocol (21). In Denmark, children aged 6–11 years and their mothers were recruited via local schools in the selected areas representing rural (Viby Sjælland) and urban (Gentofte) communities. The rural and urban areas were selected according to population density, where < 150 inhabitants/km$^2$ was defined as rural on the basis of DEMOCOPHES criteria. The parents of the children were contacted by email via school intranet and were encouraged to sign up and book an appointment through a project homepage. The following inclusion criteria were used: The child must be living primarily with the mother (> 16 days a month), and they must have been living in the same place for a minimum of 5 years, have sufficient Danish language knowledge and have normal kidney functions and must not suffer from metabolic disturbances. The goal of DEMOCOPHES was to reach 120 mother-child pairs in each country and with 75 pairs from urban area and 70 from rural area; the Danish part recruited 145 mother-child pairs in total. The children were equally distributed in gender, age and urban/rural location. All participants received detailed written information and gave consent before participating. Sampling was conducted in the urban and the rural area from September to December 2011 to minimize seasonal variation. The study was approved by the regional Ethics Committee in 2011 with supplementary approval of analyses of organophosphates including glyphosate (H-3-2011-075, H-1-2014-004). The data registry and biobank was approved by the Data Protection Agency (2011-41-6607).

## Sampling and biomarker analysis

Urine spot samples were collected in 750 mL polyethylene containers, which were delivered to the home of the participants prior to the appointment for sample collection. When arriving at the laboratory, the containers were split into smaller tubes and stored at – 20° or – 80° until analysis. The analysis of 15 different phthalate metabolites, seven parabens and nine phenols were performed at the department of Growth and Reproduction at the University Hospital of Copenhagen (12, 22). The measurement of cotinine and creatinine were performed at the Department of Environmental Medicine at the University of Southern Denmark. The measurement of cadmium was performed at the Instituto de Salud Carlos III (ISCIII) in Madrid, Spain. The analysis is described in detail in (23). Analysis of paracetamol was performed at the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, at the Ruhr University in Bochum, Germany. The analysis is described in (24, 25). Supplementary analysis of concentration of glyphosate was performed in a subsample of 27 persons selected from rural and urban locations and analyzed by Laboratory Biocheck Leipzig, Germany by the Abraxis Glyphosate Plate Kit (ELISA) according to manufacturer's protocol (26).

The collection of blood samples was a supplement in the Danish part of DEMOCOPHES. Decline to venipuncture was respected. Blood sampling was performed by trained biomedical technicians with experience in blood sampling from children. For the analysis of POPs 20 mL blood was drawn in EDTA K3 plasma tubes and centrifuged for 10 min at 2000 *g*. Plasma was transferred to 5 mL PP cryo tubes and stored in cooling box (4 °C) until arriving at the laboratory where it was stored at – 20 °C until further analysis. The plasma was analyzed for PCBs and dioxin-like activity (8), PFASs (9) and PBDEs at the University of Southern Denmark, which was also the DEMOCOPHES certified laboratory for mercury, cotinine and creatinine.

## PBDE analysis

The analysis included the following compounds: BDE-28 (2,4,4′-Tribromodiphenyl ether), BDE-47 (2,2′,4,4′-tetrabromodiphenyl ether), BDE-99 (2,2′,4,4′,5-pentabromodiphenyl ether), BDE-100 (2,2′,4,4′6-pentabromodiphenyl ether), BDE-153 (2,2′,4,4′,5,5′-hexa-bromodiphenyl ether), BDE-154 (2,2′,4,4′5,6′-hexabromodiphenyl ether) and BDE-183 (2,2′,3,4,4′,5′,6-heptabromodiphenyl ether) as well as the mass-labeled counterparts for BDE-47 (2,2′,4,4′-Tetrabromo[13C12] diphenyl ether; BDE-99 (2,2′,4,4′,5-Pentabromo[13C12]diphenyl ether), BDE-100 (2,2′,4,4′6-Pentabromo[13C12]diphenyl ether) and PBDE-153 (2,2′,4,4′,5,5′-Hexabromo[13C12]diphenyl ether); the latter used as internal standard for hexabromodiphenyl and heptabromodiphenyl ethers, were purchased from Wellington Laboratorie Inc., Guelph, ON, Canada. The quantitation of the PBDEs was performed on a Trace GC Ultra gas chromatographic system installed with a temperature programmable injector and a TSQ Quantum XLS tandem mass spectrometer operated in electronic ionization mode (Thermo Scientific, San Jose, CA, USA). The analytical separation was performed on a Rxi-5HT 15 m × 0.25 mm ID × 0.1 μm capillary column (Restek, Bellefonte, PA). Preparation of the samples by solid-phase extraction of the plasma samples was performed with two steps as described by (27). Due to PBDEs lipophilic characteristics, they were normalized to the total lipid content of the plasma sample and were reported as ng/g lipid. The total lipid content was calculated from the cholesterol and triglyceride content determined on Konelab 20 Clinical Chemistry Analyzer (Thermo Scientific, Vantaa, Finland). The precision of the method was < 8.3%, the repeatability was < 3% and the reproducibility ranged from 4.5 to 11.5%. The LOD ranged from 0.2 to 2.5 ng/mL External quality control samples from (NIST (SRM 1958 Standard Reference Material 1958 (Organic Contaminants in Fortified Human Serum))) and in-house spiked horse serum samples as well as human plasma samples was included in each series of samples analyzed. The non-fortified horse serum was purchased from Sigma-Aldrich, Munich, Germany. The accuracy of the QC samples ranged from 86 to 106%.

## Questionnaire

The basic questionnaire and the urine-sampling related questionnaire developed by DEMOCOPHES were translated into Danish. The Danish questionnaire was expanded with questions regarding exposure relevant behavior, such as living conditions in relation to traffic exposure, use of personal care products and diet. These additions were made to obtain a more detailed assessment of the exposure sources. Average biomarker results of Danish participants and the total DEMOCOPHES study were sent to the Danish participants after end analysis and individual results were provided only if participants wished to know their individual results.

## Statistics

Analysis of demographic, lifestyle, dietary and traffic-related characteristics of mothers and children were reported as numbers,

percentages or mean with range separately for urban and rural residence. For those environmental chemicals that had more than 50% values over limit of detection (LOD), the concentrations are reported as mean with range. Differences in these concentrations between mothers and their children were assessed by paired samples t-tests. Differences in concentrations of PBDEs between urban and rural residence were tested in ordinary independent sample t-tests. As the concentrations of PBDEs had a skewed distribution and had an increasing variance with higher measurements, these were ln transformed for the t-tests. The correlations between the concentrations of the different environmental chemicals were assessed, separately for mothers and children, by Spearman's rho ($\rho$). Values for the environmental chemicals measured below LOD were set to ½LOD in all statistical analyses. The urinary chemicals were corrected for creatinine, however, correction for creatinine did not change the results of the statistical analysis (results not shown).

Statistical analyses were performed in IBM SPSS statistics version 20 and the R Environment for statistical computing. The statistical significance level was set to $\alpha = 0.05$.

# Results

The characteristics of the Danish study population are shown in Table 1. The study population consisted of 145 mother-child pairs with 70 pairs from rural area and 75 pairs from urban area. The children were equally distributed in gender and age. Blood samples were obtained from 143 mothers and 123 children.

## PBDEs

The plasma samples from 143 mothers and 116 children were analyzed for PBDEs. BDE-28, BDE-47, BDE-99, BDE-100 and BDE-153 were detected in all the samples from both mothers and children, as shown in Table 2. BDE-47 was found at relatively high levels (mean of 2.68 ng/g lipid) in 143 mothers, compared to previous Danish results with a mean of 0.86 ng/g lipid in 51 pregnant mothers (16). A significantly higher level in the 116 children (mean of 3.85 ng/g lipid), compared to mothers, was found (mean of 2.68 ng/g lipid). BDE-154 was detected in 36% of the mothers and 40% of the children, while BDE-183 was only detected in 5% of the mothers and 2% of the children.

## Glyphosate

Urine samples from 13 mothers and 14 children were analyzed for glyphosate, and all samples contained detectable

**Table 1:** Characteristic of the participants in the Danish part of DEMOCOPHES.

| | Children | | | Mothers | | |
|---|---|---|---|---|---|---|
| | Urban | Rural | All | Urban | Rural | All |
| n | 75 | 70 | 145 | 75 | 70 | 145 |
| Age, mean (range) | 8.5 (6–11) | 8.5 (6–11) | 8.5 (6–11) | 42 (31–52) | 40 (31–50) | 41 (31–52) |
| Sex, n | (35♂, 40♀) | (35♂, 35♀) | (70♂, 75♀) | – | – | – |
| BMI, mean ± SD | 16 ± 1.2 | 17 ± 2.3 | 16 ± 2.3 | 22 ± 2.7 | 25 ± 5.7 | 23 ± 4.6 |
| Blood samples, n | 61 | 62 | 123 | 73 | 70 | 143 |
| Personal identity number provided, n | 57 | 49 | 106 | 61 | 50 | 111 |
| Request for personal data, n | – | – | – | 61 | 50 | 111 |
| Woodstove in house, n | 50 | 57 | 54 | 50 | 57 | 54 |
| Smokers, n | – | – | – | 6 | 12 | 18 |
| Dietary variables | | | | | | |
| Fish consumption several times a week | 36% | 39% | 39% | 57% | 30% | 44% |
| Saltwater fish consumption, weekly | 48% | 39% | 43% | 48% | 46% | 47% |
| Rice consumption, often | 43% | 31% | 37% | 69% | 71% | 70% |
| Milk consumption, daily | 76% | 83% | 79% | 75% | 70% | 72% |
| Cereal consumption, daily | 67% | 61% | 64% | 49% | 54% | 52% |
| Meat consumption, daily | 67% | 71% | 69% | 48% | 60% | 54% |
| Fast-food consumption, at least monthly | 43% | 59% | 58% | 51% | 59% | 55% |
| Cheese consumption, at least monthly | 59% | 63% | 61% | 89% | 89% | 89% |
| Traffic related variables | | | | | | |
| Window by bedroom door | 9% | 11% | 10% | 7% | 12% | 10% |
| Polluted road within 50 m of home | 17% | 33% | 26% | 17% | 33% | 26% |
| Location of work in high exposure area | – | – | – | 49% | 56% | 52% |
| Total traffic – high exposure | 19% | 36% | 28% | 11% | 25% | 19% |

BMI, body mass index.

**Table 2:** The concentrations of the environmental chemicals in the Danish part of DEMOCOPHES with significant differences between mothers and children.

| Publication | Class of chemicals | Substance | Medium | Mothers | | Children[c] | |
|---|---|---|---|---|---|---|---|
| | | | | n | Mean concentration (range) | n | Mean concentration (range) |
| Frederiksen et al. 2013 (12) | Pthalates | MEP | Urine | 145 | 74 (3.1–582) ng/mL | 143 | 28 (4.2–221) ng/mL |
| | | ∑MBP | | | 74 (5.6–501) ng/mL | | 113 (14–742) ng/mL |
| | | MBzP | | | 6.1 (0.57[a]–38) ng/mL | | 11 (0.57[a]–104) ng/mL |
| | | ∑DEHPm | | | 67(3.4–1458) ng/mL | | 99 (6.6–1815) ng/mL |
| | | ∑DiNPm | | | 24 (0.66–91) ng/mL | | 58 (2.4–2987) ng/mL |
| | Parabens | MeP | Urine | 145 | 57 (0.13[a]–955) ng/mL | 143 | 18 (0.13[a]–365) ng/mL |
| | | EtP | | | 8.4 (0.20[a]–195) ng/mL | | 1.0 (0.20[a]–25) ng/mL |
| | | *n*-PrP | | | 10 (0.09[a]–243) ng/mL | | 2.0 (0.09[a]–46) ng/mL |
| | Phenols | BPA | Urine | 145 | 4 (0.06[a]–106) ng/mL | 143 | 9 (0.06[a]–822) ng/mL |
| | | TCS | | | 66 (0.03[a]–1586) ng/mL | | 43 (0.03[a]–1065) ng/mL |
| | | BP-3 | | | 62 (0.04[a]–2442) ng/mL | | 17 (0.04[a]–885) ng/mL |
| | | 2,4-DCP | | | 1.00 (0.04[a]–75) ng/mL | | 0.58 (0.04[a]–5.5) ng/mL |
| | | 2,5-DCP | | | 0.67 (0.04[a]–15) ng/mL | | 0.78 (0.04[a]–19) ng/mL |
| Mørck et al. 2014 (8) | PCBs | Total PCB[b] | Blood | 143 | 0.17 (0.05–0.69) µg/g lipid | 116 | 0.13 (0.01–0.63) µg/g lipid |
| Mørck et al. 2015a (9) | PFASs | PFOA | Blood | 143 | 1.80 (0.35–8.19) ng/mL lipid | 116 | 3.20 (1.40–6.65) ng/mL lipid |
| | | PFHxS | | | 0.39 (0.08–1.74) ng/mL lipid | | 0.44 (0.015[a]–3.68) ng/mL lipid |
| | | PFNA | | | 0.75 (0.26–2.55) ng/mL lipid | | 0.88 (0.28–2.16) ng/mL lipid |
| | | PFDA | | | 0.33 (0.08–1.14) ng/mL lipid | | 0.34 (0.11–0.75) ng/mL lipid |
| | | *total*-PFOS | | | 8.30 (2.52–24.38) ng/mL lipid | | 9.02 (2.77–23.01) ng/mL lipid |
| Mørck et al. 2015b (10) | Mercury | | Hair | 145 | 560 (9.9–2822) ng/g | 144 | 326 (9.7–1.335) ng/g |
| | Cadmium | | Urine | 142 | 0.17 (0.01[a]–1.09) µg/L | 142 | 0.03 (0.01[a]–0.27) µg/L |
| | Cotinine | | Urine | 143 | 0.15 (0.00[a]–3.40) µg/L | 144 | 0.001 (0.00[a]–0.02) µg/L |
| Nielsen et al. 2015 (14) | Paracetamol | NAAP | Urine | 145 | 64250 (49–3,037,000) µg/L | 143 | 29.91 (2.79–2,257,000) µg/L |
| Not in previous publication | PBDEs | BDE28 | Blood | 143 | 0.21 (0.08–1.09) ng/g lipid | 116 | 0.24 (0.07–1.16) ng/g lipid |
| | | BDE47 | | | 2.68 (0.81–18.55) ng/g lipid | | 3.85 (1.09–40.66) ng/g lipid |
| | | BDE99 | | | 1.85 (0.35–16.20) ng/g lipid | | 2.29 (0.38–31.35) ng/g lipid |
| | | BDE100 | | | 0.58 (0.136–3.01) ng/g lipid | | 0.85 (0.14–6.55) ng/g lipid |
| | | BDE153 | | | 1.96 (0.46–28.70) ng/g lipid | | 1.39 (0.21–7.74) ng/g lipid |
| Not in previous publication | Glyphosate | | Urine | 13 | 1.28 (0.49–3.22 ng/mL | 14 | 1.96 (0.85–3.31) ng/mL |

levels of glyphosate (See Table 2). There was no discernable difference between urban and rural residences.

## All biomarkers

All of the 66 environmental chemicals were analyzed in the available samples, with blood samples available for only 116 of 145 children due to refusal of blood sampling. 23 chemicals were found in 100% of the mothers, while 22 chemicals were found in 100% of the children, with significant overlap of the chemicals found in both groups. Forty-eight of the biomarkers measured were detected in over 50% of the mothers, while 42 were detected in over 50% of the children. In Table 2, we have summarized a selection of biomarkers.

## Mother-child differences

Table 2 shows the concentrations in mothers and children for a selected number of chemicals and classes of chemicals with significant differences between mothers and children. Children had higher concentrations of phthalates except Mono-ethyl phthalate (MEP) as also seen in DEMOCOPHES (2). Parabens and phenols were measured in higher concentrations in mothers compared to children, except BPA, which was also measured in higher concentration in children compared to mothers in the six DEMOCOPHES countries (7). Urine biomarkers of cadmium, cotinine, and paracetamol were significantly higher in mothers, while glyphosate was significantly higher in children. Serum concentrations of total PCBs and BDE-153 were higher in mothers compared to children while all Perfluorooctane sulfonate (PFOS) and BDE 28, 47, 99 and 100 were higher in children. The mother-child correlations were assessed for all biomarkers, and significant correlations were found for PCB 28 and 156, $\beta$-hexachlorocyclohexane (β-HCH), *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and aryl hydrocarbon receptor mediated TCDD equivalence (pg TEQ/g lipid) (AhR-TEQ), all measured PFASs, mercury in hair, cotinine in urine as well as MN frequency and the PBDEs 28, 47, 100, and 153. All of the measured non-persistent substances correlations between mothers and children were found as previously reported.

Analysis of differences between living area and levels of the PBDEs showed significant higher levels of BDE-99, BDE-100 and BDE-154 in mothers from rural area and significant higher levels of BDE-28 in mothers from urban area. For children, the only significant differences were found in levels of BDE-99 and BDE-153, where BDE-99 was

**Table 2** (continued)

[a]< LOD is set to ½LOD. [b]Total PCB = (PCB153 + PCB138 + PCB180)*2. 'significant difference between mothers and children. ∑MBP(i + n), the sum of MnBP and MiBP; ∑ DEHPm, the sum of DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP), ∑DiNPm, the sum of DiNP metabolites (MiNP, MHiNP, MOiNP and MCiOP). Biomarkers not found in any participants: mono-iso-decyl phthalate (MiDP), i-butylparaben (i-BuP), o,*p′*-dichlorodiphenyldichloroethylene (o,*p′*-DDE). Biomarkers only found in < 50% of the participants: mono-*n*-butyl phthalate (MOP), mono(3-carboxypropyl) phthalate (MCPP), triclocarban, 2,4,5-trichlorophenol (2,4,5-TCP), 2-phenylphenol (2-PP), 4-phenylphenol (4-PP), i-propylparaben (i-PrP), n-butylparaben (n-BuP), benzylparaben (BzP), 2,2′,4,4′5,6′-hexabromodiphenyl ether (BDE154), 2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE183), o,*p′*-dichlorodiphenyltrichloroethane (o,*p′*-DDT). Except for cotinine and cadmium that are reported in the table, due to their specific nature, and due to these being a part of the international DEMOCOPHES project. For the PCBs: only the PCBtotal is reported due to the fact that around 50% of the PCBs in humans consist of the three PCBs 153, 138, 180 (28). In addition, these three PCBs are found in 99–100% of all the participants in the present study. MEP, Mono-ethyl phthalate; ∑MBP(i + n), the sum of mono-*n*-butyl phthalate (MnBP) and mono-iso-butyl phthalate (MiBP); MBzP, monobenzyl phthalate; ∑DEHP, the sum of the di-(2-ethylhexyl) phthalate metabolites; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; and MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; ∑DiNP, the sum of the di-iso-nonyl phthalate metabolites; MiNP, mono-isononyl phthalate; MHiNP, mono(hydroxyl-iso-nonyl) phthalate; MOiNP, mono(oxo-iso-nonyl) phthalate; and MCiOP, mono(carboxy-iso-octyl) phthalate; MeP, methylparaben; EtP, ethylparaben; n-PrP, n-propylparaben; BPA, bisphenol A; TSC, triclosan; BP-3, benzophenon-3; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; PCBs, polychlorinated biphenyls; OCPs, organochlorine pesticides; HCB, hexachlorobenzene; *p,p′*-DDT, *p,p′*-dichlorodiphenyltrichloroethane; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene; AhR-TEQ, aryl hydrocarbon receptor mediated TCDD equivalence (pg TEQ/g lipid); PFASs, polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFOS, perfluorooctane sulfonate; NAAP, N-acetyl-4-aminophenol; PBDEs, polybrominated diphenyl ethers; PBDE-28, 2,4,4′-tribromodiphenyl ether; PBDE-47, 2,2′,4,4′-tetrabromodiphenyl ether; PBDE-99, 2,2′,4,4′,5-pentabromodiphenyl ether; PBDE-100, 2,2′,4,4′6-pentabromodiphenyl ether; PBDE-153, 2,2′,4,4′,5,5′-hexabromodiphenyl ether.

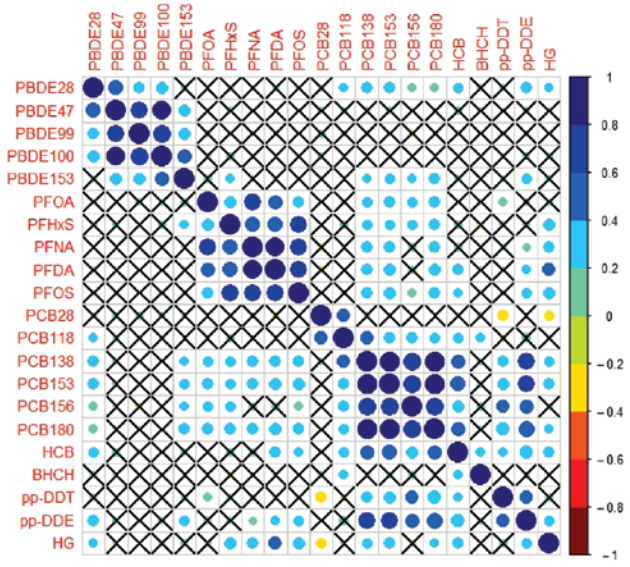


**Figure 1:** Correlation matrix for the POPs and mercury in children in the Danish part of DEMOCOPHES.
The figure shows the correlations between the measured POPs and mercury in children (n = 116). The color and size indicates the strength of the correlation. The color scale is shown on the right. A cross symbolizes that there is no significant correlation. Significance level was set to 0.05.

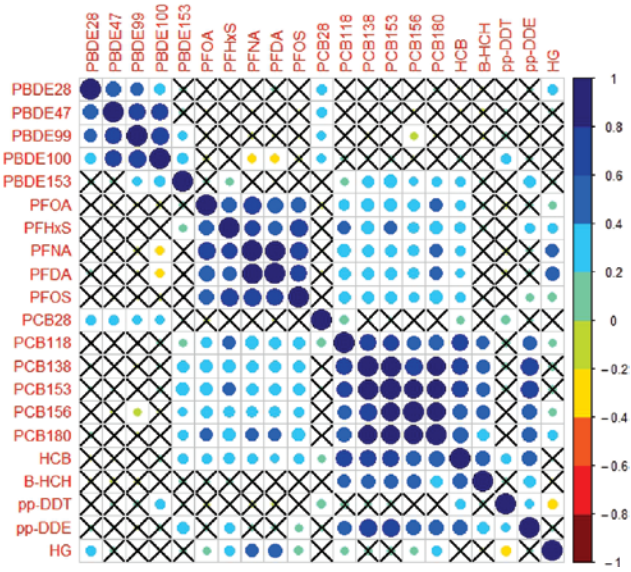


**Figure 2:** Correlation matrix for the POPs and mercury in mothers in the Danish part of DEMOCOPHES.
The figure shows the correlations between the measured POPs and mercury in mothers (n = 143). The color and size indicates the strength of the correlation. The color scale is shown on the right. A cross symbolizes that there is no significant correlation. Significance level was set to 0.05.

higher in rural area, and BDE-153 was higher in urban area (results not shown).

### Correlation between environmental chemicals

Significant correlations were found between nearly all chemicals within the same class. Figures 1 and 2 shows, for mothers and children, respectively, the correlations within the same class and in the different groups of POPs and mercury.

For the non-persistent urinary biomarkers, moderate but statistically significant correlations were found between several of the chemicals from each class of phthalates, parabens and phenols, especially in children where MEP were significantly correlated with BPA, triclosan, benzophenone-3 and 2,4-dichlorophenol [2,4-DCP ($\rho$ : 0.19–0.40, $p < 0.05$)] and BPA was correlated with the phthalates MBzP, diethylhexyl phthalate (DEHP) and Di-iso-butyl phthalate [DiNP ($\rho$ : 0.17–0.27, $p < 0.05$)]. In mothers, 2,5-DCP was correlated with the parabens ($\rho$ : 0.2–0.22, $p < 0.05$) (12).

Finally, the correlations between the urinary, blood and hair biomarkers were tested, and no consistent pattern of correlations between whole classes of chemicals was observed, but there were a few sporadic significant correlations (29).

## Discussion

### PBDEs

For four out of five of the PBDEs, significantly higher levels were detected in children, in all of the children and adult samples. The higher levels detected in children may be partly due to the relatively small body size and blood volume of children relative to their ingestion, leaving them with smaller body storage for the compounds. Furthermore, the exposure burden may be larger in children compared to adults, because of immature metabolic pathways in children (30). The higher concentrations found in children may also be due to different sources of exposure in mothers and children, or that children are exposed to a higher degree than their mothers. Although the children in this study are not toddlers, it is likely that they get into more contact with floor dust than their mothers, causing

higher exposure to the PBDEs. A study of exposure in Danish newborns and mothers showed clear correlation between maternal PBDE serum levels and dust PBDE (31). That study also found a positive correlation between number of electronic devices in the homes and PBDE in the mothers (unpublished).

Higher levels for several PBDEs in mothers as well as children in rural areas, may be due to house construction in the 1960s using carpets and other constructions with PBDEs, consistent with higher rural PCB28 levels from sealing materials used in these houses (13). A subsample of mothers and 3-year-old children from the Norwegian Mother Child Cohort 1999–2005 showed lower concentrations in Norway compared to Danish levels (32). The Danish levels in children were lower than concentrations reported in National Health and Nutrition Examination Survey (NHANES) 2003–2004 (33), and in the studies of 9–12-year-old children in California in 200–2002 (34). In addition, levels in adult Danish females were lower compared to the adult pregnant women in NHANES 2003–2004 (35).

## Glyphosate

Glyphosate concentrations in the Danish sample of 13 mothers and 14 children are in line with the German study ("Urinale 2015"), which detected glyphosate residues in 99.6% of the spot urine samples from 2011 participants, using the same analytical method (Abraxis-ELISA-test method, with LOD 0.0751 μg/L). The mean value for all samples was 1.08 ng/mL, with a maximum value of 4.2 ng/mL. The youngest participants in the German study – with participants ranging from 0 to 9 years and 10 to 19 years – had the highest concentrations of glyphosate in the urine (36, 37). Further unpublished studies of 24 adult Danes, using the same analytical method confirmed the level of 1 ng/mL. Conrad et al. (38) analyzed archived samples from 2001–2015 provided by 20 to 29-year-old individuals living in Greifswald, a city in north-eastern Germany. Out of the 399 analyzed urine samples, 127 (31.8%) contained glyphosate concentrations at or above the limit of quantification (LOQ) of 0.1 μg/L. Male donors had the highest levels and an increase over time was observed.

In 2013, 182 human spot urine samples from 18 European countries were tested for glyphosate and its metabolite aminomethylphosphonic acid (AMPA) (GC-MS/MS method with LOQ 0.15 μg/L). On average, 44% of the samples were above the limit of quantification (LOQ) (0.15 μg/L), but the frequency of detection across countries varied from 10% to 90%. The highest glyphosate concentration was 1.82 μg/L from a Polish sample (the highest AMPA 2.6 μg/L, sample from Croatia) (39).

In a study of 26 Portuguese volunteers from an urban area conducted by the 'Portuguese No GMO Coalition', glyphosate was detected in all samples in a range from 12.5–32.5 ng/mL, with an average amount of 26.2 ng/mL, which is much higher than levels in German and Danish studies (LC-MS/MS method with LOQ 0.5 ng/mL) (40).

In Denmark, glyphosate residues have been found in, e.g. barley, wheat grain, wheat flour, oat grain, cornflakes, dried lentils, and dried chickpeas, either from Danish or foreign origin (41, 42), all below the current maximum residue level (MRL). Assuming 20–30% uptake from food and no metabolism, these values are substantially below the acceptable daily intake (ADI) and acute reference dose (ARfD) set by the European Food Safety Authority (EFSA) (43) in 2015 to 0.5 mg/kg body weight/day, with the acceptable operator exposure level (AOEL) at 0.1 mg/kg body weight/day (EFSA, 2015). However, the possible carcinogenic potential of glyphosate is discussed by the International Agency on Research of Cancer (IARC), which has classified glyphosate as a probable carcinogen (group 2A) (44), and the EFSA, which states that there is no carcinogenic risk from oral exposure to glyphosate residues in food (ref). Information about human absorption, metabolism and excretion rate (half-life) is very scarce, with only one human study reporting apparent elimination half-life around 3.1 h (45) versus half-lives of 33 h reported for rodents (44). If the half-life is as short as 3.1 h, the sampling time becomes critical in interpretation of exposures.

## All biomarkers in the study

The specific sources of exposures were only found in few compounds, that have been discussed in previous publications. Mercury concentrations in hair have been associated with fish consumption as described previously in Mørck et al. (10), which has also been confirmed in DEMOCOPHES (2, 23) and in other studies (46–49).

The source of PCB 28 was investigated further in the Danish participants, and concentrations of PCB 28 were significantly higher in both mothers and children living in houses built in the period (1950–1977), when PCBs were used in construction and sealants in houses to a higher degree than houses built earlier (13).

Six DEMOCOPHES countries found a significant association between BPA and consumption of canned food in mothers (7), which was also found in Spanish pregnant

women (50). The use of personal care products by mothers was associated with higher concentrations of phthalates and parabens (12). All these findings support the hypothesis of lifestyle- and dietary-related exposures, even to non-persistent chemicals.

The was significant positive correlation between concentrations of some persistent chemicals and metals to the age of the mothers in the present study. The increase in concentration of persistent chemicals correlated to increasing age of adults is consistent with previous studies and expectable, as accumulation of persistent chemicals in humans increase over time (51–57).

Significant correlations were found for all POPs within the same class, as reported also in the literature. (55, 58–60), Simultaneous exposure to chemicals within the same class indicates that the compounds are either used together in the same products, or that similar products are often used by the same consumers. The presence of persistent chemicals is a result of many years of exposure, accumulation, and metabolism of the chemicals. Nearly all compounds measured in the present study can be categorized as endocrine disruptors, some of which have been suspected to affect the reproductive system in males, as well as play an important role in the decline of semen quality and increase in testicular cancer in Denmark (61–63). Effects on reproduction in females have also been reported for compounds measured in the present study (64–67). Furthermore, neurological and immunological effects have been associated to chemical exposure (28, 58, 68, 69).

Bonefeld-Jørgensen et al. (70) emphasizes the need for both epidemiological and in vitro/ex vivo studies to investigate the potential impact of the effect of multiple POPs on human health. The most efficient way to prevent these health effects is to reduce exposure by increasing regulation with regards to limiting the chemical content in consumer products, and issuing public recommendations on diet and other exposure-related habits.

The strength of analyzing many different biomarkers is that it provides a more detailed exposure map, as well as the possibility of elucidating exposure patterns between the different classes of chemicals. However, it can also be an advantage to limit the number of congeners in each chemical class, or merge some of the congeners, to represent the overall chemical for both practical and economic reasons.

The mother-child correlations found in this study may be due to the influence mothers have over their children at this age, and the choice their mothers make regarding main household products used. In a German study, this exposure pattern was found for phthalates and BPA (71).

The questionnaire is based on the DEMOCOPHES questionnaire used in all participating countries. As ingestion is the primary source of many chemicals, a more detailed food frequency questionnaire may facilitate the identification of specific sources.

The cross-sectional design of this study limits the reproducibility of the individual measurements of non-persistent chemicals, as there may be day-to-day variation dependent on the exposure within the last 24 h, due to short half-lives (6). The average data is however very useful in estimating the general exposure levels.

## Conclusion

In conclusion, the exposure to chemicals in the Danish participants of the present study is ubiquitous.

The correlations between the measured environmental chemicals indicate that there may be a specific exposure pattern, where people who are highly exposed to one class of environmental chemicals could also experience high exposure to certain other classes. As some of the compounds were measured at higher levels in children compared to mothers, increased focus on the exposure in young children is recommended. For a more detailed investigation of specific exposure sources, more studies with increased power and detailed questionnaires should be developed.

With the abundant simultaneous exposure to many chemicals, there is a risk of adverse health effects, with specific emphasis on the exposure of children, since they are sensitive to exogenous stimuli. Furthermore, many of the mothers are of a fertile age, placing increased importance on limitation of exposure during pregnancies.

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detailed written information and gave consent before participating. Ethical approval: The study was approved by the regional Ethics Committee in 2011 with supplementary approval of analyses of organophosphates including glyphosate (H-3-2011-075, H-1-2014-004). The data registry and biobank was approved by the Data Protection Agency (2011-41-6607).

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