Exposure to multiple chemicals in a cohort of reproductive-aged Danish women

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Abstract

Background—Current exposure assessment research does not sufficiently address multi-pollutant exposure and their correlations in human media. Understanding the extent of chemical exposure in reproductive-aged women is of particular concern due to the potential for *in utero* exposure and fetal susceptibility.

Objectives—The objectives of this study were to characterize concentrations of chemical biomarkers during preconception and examine correlations between and within chemical classes.

Methods—We examined concentrations of 135 biomarkers from 16 chemical classes in blood and urine from 73 women aged 18–40 enrolled in Snart Foraeldre/Milieu, a prospective cohort study of pregnancy planners in Denmark (2011–2014). We compared biomarker concentrations with United States similarly-aged, non-pregnant women who participated in the National Health and Nutrition Environmental Survey (NHANES) and with other international biomonitoring studies. We performed principal component analysis to examine biomarker correlations.

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Results—The mean number of biomarkers detected in the population was 92 (range: 60–108). The most commonly detected chemical classes were phthalates, metals, phytoestrogens and polycyclic aromatic hydrocarbons. Except blood mercury, urinary barium and enterolactone, geometric means were higher in women from NHANES. Chemical classes measured in urine generally did not load on a single component, suggesting high between-class correlation among urinary biomarkers, while there is high within-class correlation for biomarkers measured in serum and blood.

Conclusions—We identified ubiquitous exposure to multiple chemical classes in reproductive-aged Danish women, supporting the need for more research on chemical mixtures during preconception and early pregnancy. Inter- and intra-class correlation between measured biomarkers may reflect common exposure sources, specific lifestyle factors or shared metabolism pathways.

Keywords

Environmental exposures; NHANES; Biomonitoring; Principal component analysis; Pregnancy; Preconception

1. Introduction

In addition to evaluating the impact of exposure to *individual* chemicals on human health, new studies are beginning to jointly assess the effects of chemical *mixtures* (Braun et al., 2016; National Institute of Environmental Health Sciences [NIEHS], 2015a; Rider et al., 2013; Wild, 2005). Biomarkers of exposure assessed by biomonitoring (i.e. parent compounds and/or metabolites measured in biological media such as blood or urine) are typically considered one of the best measures to characterize the total amount of a chemical absorbed via multiple routes. However, the feasibility of collecting biomonitoring data on multiple chemicals within an individual is limited by the high cost of analysis and by statistical complexity of analyzing and interpreting results. Thus, most investigations are limited to either individual biomarkers or multiple biomarkers within the same class, such as metals, polychlorinated biphenyls (PCBs) or per- and polyfluoroalkyl substances (PFAS) (Cupul-Uicab et al., 2013; García-Esquinas et al., 2011; May et al., 2015; Sanders et al., 2015).

In an effort to characterize the exposome, or the totality of non-genetic exposures that a person experiences from conception until death along with the associated biological response, assessing biomarkers of exposure among women of childbearing age can help quantify a mother's contribution to their child's exposome during sensitive developmental periods (National Institute of Environmental Health Sciences [NIEHS], 2015b; Robinson and Vrijheid, 2015; Wild, 2005). Many chemical analytes within the same usage or chemical class are densely correlated (Rappaport, 2012; Robinson et al., 2015; Sun et al., 2013). The extent to which women of childbearing age are exposed to multiple chemicals and how these biomarkers of exposure correlate with each other is currently an active area of research (Agay-Shay et al., 2015; Patel and Ioannidis, 2014; Robinson et al., 2015; Woodruff et al., 2011). Analyzing correlations between biomarkers among women of childbearing age has several advantages: allow future investigators to focus on shared exposure sources, identify

chemicals that may travel together in the body and observe which chemicals are likely to co-occur in order to direct research towards mixtures to which women of childbearing age are actually exposed.

Investigators have suggested some statistical approaches to quantify correlations between measured biomarkers of exposure. Most of these methods contain a data reduction component, whereby the magnitude of correlation between biomarkers identifies a new set of independent comparisons that is less than the number of biomarkers assessed. Principal Component Analysis (PCA) is one such method used to quantify correlations between exposures in the exposure assessment literature (Allen et al., 2008; Herrick et al., 2007; Robinson et al., 2015). PCA identifies the maximum amount of mutual correlation between groups of variables that explain latent variables, or components, that cannot be directly observed (Burstyn, 2004). Biomarkers are categorized under a given component based on their "loading," which represents correlations between the biomarker and the underlying, latent factor, or component. Because the components are orthogonal, or statistically independent, biomarkers loaded within one component are said to have a low correlation with biomarkers loaded on all other components. The extent to which chemicals load to the final components may indicate common exposure sources within that component, such as chemicals that are found together in diet, consumer products or traffic pollution. Loading patterns may also suggest chemical biomarkers that are found at consistently high or consistently low concentrations among participants. PCA results can serve as a guide to confounder/co-exposure adjustment in statistical modelling for investigators evaluating associations between health outcomes and one or many chemical classes. PCA results may also identify the extent to which chemical exposures share common sources and pathways or jointly contribute to disease. While PCA has been commonly used in the occupational health literature, few investigators have used PCA in exposure assessment in women of childbearing age (Robinson and Vrijheid, 2015).

In the current study, we assessed preconception levels of 135 chemicals and/or their metabolites in the blood, serum and urine of 73 Danish women aged 18–40 participating in a prospective cohort study of pregnancy planners (2011–2014). These included metals, persistent chemicals, such as organochlorines, and non-persistent chemicals, such as phthalates, phytoestrogens and pesticides. We compared their concentrations with those of U.S. women of child-bearing age who participated in NHANES 2005–2012 and with other international biomonitoring studies (Cerna et al., 2012; Den Hond et al., 2015; Fréry et al., 2012; Haines and Murray, 2012; Kolossa-Gehring et al., 2012; Mørck et al., 2015b; Perez-Gomez et al., 2013; Schoeters et al., 2012). We used PCA to quantify the correlation of biomarkers between and within chemical classes. Unlike previous biomonitoring studies that only report on one or a few chemical classes, we measured concentrations of several chemical classes within the same individuals. We also reported on correlation patterns to gain a more thorough understanding of common co-exposures and the "pregnancy exposome."

2. Methods

2.1. Study design

The current biomonitoring study, Snart Foraeldre/Milieu, English translation-'Soon Parents/ Environment', is a sub-study of Snart Foraeldre (SF). SF is a prospective cohort study of Danish women from the general population who stopped using contraception because they wished to become pregnant (Huybrechts et al., 2010; Mikkelsen et al., 2009). All study recruitment and follow-up in the parent study, SF, is conducted via the Internet (www.snartforaeldre.dk). Participants for the study were recruited through advertisement on a popular Danish health-related website (www.netdoktor.dk), social media (e.g. Facebook), blogs and word of mouth. SF is a continuation of a previous study, Snart Gravid, for which study methods have been described in detail previously (Hatch et al., 2016; Huybrechts et al., 2010; Mikkelsen et al., in press, 2009; Wise et al., 2009).

Briefly, women eligible for SF are Danish residents, aged 18–40 years, in a stable relationship with a male partner, and not currently using any form of contraception or fertility drugs. Eligibility is assessed via an online screening questionnaire and participants complete an online consent form before enrollment. To enroll, women are required to provide a valid Civil Registration Number (CPR) and e-mail address. Informed consent was obtained by each participant over the Internet. The CPR number is used for linkage to Danish registries, such as the Danish Medical Birth Registry and the Danish National Registry of Patients. After completing an extensive baseline questionnaire, participants are followed via short bimonthly questionnaires until pregnancy is reported or for up to one year. A total of 3500 women have enrolled in SF since August 2011.

To enroll in the biomonitoring sub-study, SF/Milieu, women had to meet the above eligibility requirements and also live within the city limits of Aalborg, located in Northern Jutland. A total of 223 women from Aalborg enrolled in SF between November 2011 (when SF/Milieu study began) and December 2014. After completing the baseline questionnaire, they were invited to participate in the Milieu study. A total of 129 women (58%) agreed to participate in SF/Milieu. Women who enrolled in the study visited the clinical research laboratory at Aalborg University Hospital for blood and urine collection during the preconception period, and if pregnancy occurred, during the 1st (~10 weeks) and 2nd (~20 weeks) trimesters.

For the purposes of this study, we were interested in measuring chemical concentrations in women only during the preconception time period. At the time of this study, we collected biospecimen samples from 73 women in the first preconception visit. Of those 73, results from 16 urine samples were not reported due to improper aliquot mixing in the field. Therefore, the number of participants included in the analysis varied, with a total of 56 urine and 73 serum samples.

The SF/Milieu study was approved by the Danish Data Protection Board (2006-41-6864), the North Denmark Region Committee on Health Research Ethics (N-20100087) and the Institutional Review Board at Boston University. The analysis of blinded specimens by the

Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects' research.

2.2. Biospecimen collection

Blood and urine specimens were collected using materials provided by the CDC, in accordance with protocols used for biomonitoring in NHANES (Centers for Disease Control and Prevention [CDC], 2014). We collected samples within one month of filling out the baseline questionnaire. After signing a written informed consent for blood and urine collection, participants reported time of last meal and date of last menstrual period. A trained phlebotomist first drew 3 mL aliquots of whole blood, which was immediately frozen for future quantification of metals. The remaining blood was collected in three 10 mL tubes (approximately 9 mL each) and allowed to clot for 2 h. After centrifuging, 4–5 mL of serum were transferred to 10 mL glass vials for measurement of brominated flame retardants (BFRs). Additional centrifuging was performed as necessary if red blood cells mixed with serum, and the remaining serum was transferred to Simport cryovials and frozen at −80 °C to be shipped to the CDC.

Polypropylene collection cups were provided to the participant for urine collection. Soon after receipt of the urine specimen, a trained technician prepared one aliquot for speciated arsenic and the remaining urine was frozen in the collection cup. Urine samples were frozen at −20 °C until shipped to the CDC where they were divided into eight more aliquots for analysis of the remaining chemical classes to be measured in urine.

2.3. Chemical analysis

Blood, serum and urine specimens were analyzed at the National Center for Environmental Health (NCEH) laboratories (CDC, Atlanta, GA). We chose chemicals for analysis if they were currently being measured as part of NHANES. Biomarkers measured in urine included: fifteen heavy and trace metals, seven arsenic species, ten polycyclic aromatic hydrocarbon (PAHs) metabolites, five phenols, four parabens, twelve phthalate metabolites, five diakylphosphate (DAP) metabolites, two herbicides, six organophosphate and pyrethroid insecticides metabolites, N,N-diethyl-meta-toluamide (DEET) and three metabolites, six phytoestrogens and perchlorate. Biomarkers measured in serum include: eleven BFRs, eight organochlorine pesticides, twenty-four PCBs, eight PFAS, and cotinine. We also measured five metals in whole blood.

We measured cadmium, lead and manganese in both blood and urine to maintain consistency with the NHANES panel and to provide flexibility for future epidemiological studies that wish to examine relationships between metals and outcomes with long or short latency periods. For instance, blood cadmium has been shown to reflect recent exposure, whereas urinary cadmium more accurately reflects long-term exposure (Adams and Newcomb, 2014). In addition, metals concentrations in different media have been shown to be differentially mediated by age and sex (Adams and Newcomb, 2014). Metals detected in both blood and urine are reported as independent due to their uncorrelated nature in both media (Adams and Newcomb, 2014; Gulson et al., 1998; Moreira and Neves, 2008). Similarly, total arsenic and arsenic metabolites are reported as independently detected

because arsenic metabolites were not consistently detected in study participants, and to maintain consistency with the NHANES panel.

A total of 135 biomarkers were measured in blood, serum or urine. Table 1 summarizes the number of biomarkers measured in each media and the laboratory protocols used for each chemical class. The biomarkers measured in this study broadly overlapped with those currently measured in NHANES. CDC NHANES Laboratory Method Manuals may be found on the CDC website (http://wwwn.cdc.gov/nchs/nhanes/search/DataPage.aspx?Component=Laboratory), which includes information about analysis procedures and use of laboratory blanks during sample analysis.

2.4. Statistical analysis

We compared descriptive statistics for biomarkers in SF/Milieu and NHANES populations. Publicly available data from NHANES are released in two-year cycles and include demographic characteristics and laboratory measurements for select biomarkers in blood, serum and urine. The list of select biomarkers changes with each data release. Therefore, we analyzed data from NHANES 2005–2006, 2007–2008, 2009–2010, and 2011–2012, to cover the complete set of biomarkers measured in SF/Milieu. We restricted the NHANES analysis to non-pregnant women between the ages of 18 and 40 to match the Danish study population characteristics. Data for organochlorine pesticides were only available in weighted serum samples prepared from one-third of the NHANES 2007–2008 population, pooled by gender (male, female), race and ethnicity (non-Hispanic white, non-Hispanic black, Mexican American) and age groups (12–19, 20–39, 40–59, and 60 years of age and older). Therefore, organochlorine pesticide data were restricted to women 20–39 years old. To account for the NHANES complex sample design, we used PROC SURVEY in SAS to calculate descriptive statistics (Centers for Disease Control and Prevention CDC, 2013).

We replaced concentrations below the limit of detect (LOD) with the LOD divided by the square root of two (Hornung and Reed, 1990). For most chemicals, the LOD is constant across samples. However, for polybrominated diphenyl ethers (PBDEs), PCBs, and organochlorine pesticides, each individual sample has its own LOD based on sample volume analyzed. For these chemicals, we reported the maximum LOD. We present descriptive statistics (range, geometric mean, detection frequency) for biomarkers that were detected in over 60% of the SF/Milieu samples.

We then performed principal component analysis (PCA) using PROC FACTOR (SAS version 9.3) in the SF/Milieu population to identify correlated biomarkers. After omitting the 16 participants with compromised urine samples, 1.5% of additional biomarker values were marked as missing due to insufficient sample volume. Because PCA omits individuals with any missing variables, we imputed missing values using multiple imputation by the Markov Chain Monte Carlo multivariate normal model (Schafer, 1997). Data were log-transformed within the imputation procedure to fulfill the normality assumption. We assessed the missing at random assumption by examining missing data patterns. This approach is appropriate for models that assume arbitrary missing, continuous data, such as PCA (van Ginkel and Kroonenberg, 2014). We imputed 15 datasets as recommended by Graham et al. (2007). Variables included as predictors of missing observations can be found in Supplemental Files.

Imputed negative values were replaced with the biomarker LOD divided by the square root of 2.

We performed PCA on concentrations of creatinine-adjusted biomarkers measured in urine, volume-based blood metals and PFAS, and lipid-adjusted PBDEs, PCBs and persistent pesticides. We created three models for the PCA: all biomarkers combined (Model 1), urinary biomarkers only (Model 2) and serum and whole blood biomarkers only (Model 3). We restricted the analysis to biomarkers that were detected in 75% of the study population to enhance reliability in the PCA estimates. We used two methods to determine the optimal number of principal components to output for each model. These included visually examining the scree plot, an output of PCA analysis, which displays the component numbers on the x-axis and the eigenvalues, or the amount of variability explained by each component, on the y-axis and examining the proportion of variability in the data explained by each component. Additionally, we found that after three components for Model 1, and two components for Models 2 and 3, the proportion of variance explained by each additional component was due primarily to only one or a few biomarkers. Based on these considerations, we restricted Model 1 to three principal components and Models 2 and 3 to two components. We considered factor loadings greater than 0.25 to indicate a high loading.

We performed PCA separately on each imputed dataset and then averaged eigenvalues (a measure of the amount of variance explained by each biomarker along a given principal component) and component scores to determine analyte loadings on each component. We applied a varimax rotation due to the uncorrelated nature of the factor patterns. This step maximizes the sum of squared correlations between each biomarker and component, which simplifies the interpretation of each component. Using the PCA results, we examined biomarker correlations within each extracted component.

To examine if the loading patterns differed between imputed and non-imputed datasets, we performed PCA in 1) all 15 imputed datasets 2) one of the 15 imputed datasets 3) the original, unimputed data. We performed all statistical analyses in SAS (version 9.3; SAS Institute Inc., Cary, NC).

3. Results

Table 2 presents study population characteristics. The majority of the study population (n=73) was highly educated and either employed or a student (88%), with a median age of 28 years, (IQR 25–30.5). Participants were asked if they were regularly (daily or almost every day) exposed to pesticides, metal particulates, solvents, engine exhaust or cosmetic chemicals during work or home activities. A total of 13% of women reported being exposed to chemicals through their occupation, and 26% reported exposure to engine exhaust. About half of the population made below 39,999 Danish Krone per month ($5826 USD). Over half the women in the population were nulliparous, and most women had been trying to conceive between 1 and 3 months at enrollment. The population characteristics outlined in Table 2 are similar between the parent (SF) and this sub-study (Milieu) populations (data not shown). For example, both populations are highly educated, where 33% and 39% of the full SF and SF/Milieu populations, respectively, have advanced degrees. Similarities can also be found

in employment status. In both the parent SF and Milieu population, greater than 50% are working and less than 10% are unemployed. Similarly, in the parent SF population, 27% are student and 34% are students in the Milieu population. Average age and BMI are almost identical in the two populations. A slightly higher percentage of participants (54%) have a monthly income greater than 39,999 DKK in the parent SF study compared Milieu participants (47%).

Fig. 1 presents the number of chemical biomarkers detected in the 56 participants who had the complete set of chemical biomarkers measured in blood, serum or urine. Out of 135 measured biomarkers, the highest number detected in any individual was 108, and the lowest number was 60. The mean number of chemicals detected was 92 and the median was 95. Because lead, manganese and cadmium were measured in both blood and urine, Fig. S2 in the Supplemental Material presents the number of chemical biomarkers detected, with urinary lead, urinary manganese and blood cadmium omitted. By omitting these measures, the mean, minimum and maximum number of chemicals detected is 89, 58 and 105 respectively. Seven participants had cadmium detected in blood, but not in urine, one participant had lead detected in urine but not blood, and zero participants had cadmium detected in urine but not blood.

The following biomarkers were detected in 100% of participants: phytoestrogens (enterodiol, enterolactone, genistein), PCBs (PCB74, PCB 138–158, PCB 153, PCB180, PCB187), hexachlorobenzene (a persistent pesticide), perchlorate, PFAS (perfluorodecanoate, perfluorohexane sulfonate, perfluorononanoate, perfluorooctoanoate, perfluorooctane sulfonate), urinary metals (total arsenic, barium, thallium, strontium, molybdenum, cesium, cobalt), blood metals (manganese, lead, and selenium), and PAH metabolites (9-hydroxyfluorene, 2-hydroxynaphthalene and 2-hydroxyfluorene). With the exception of PBDE153, PBDEs were detected in less than 20% of the participants. Of the nine measured phenols, only bisphenol A (BPA), benzophenone-3, methyl paraben and propyl paraben had high detection frequencies (95%, 86%, 96% and 86% respectively). Triclosan was detected in only 52% of the population. Less than 30% of participants had detectable levels of most types of pyrethroids, DEET metabolites, and organophosphate (OP) pesticide metabolites. Among the pyrethroids, DEET and OP classes, only diethylphosphate and dimethylthiophosphate (DAP metabolites), 3-phenoxybenzoic acid (a non-specific pyrethroid metabolite) and para-nitrophenol (a metabolite of OP insecticides) were commonly detected (> 90%).

Table 3 presents concentrations of biomarkers in the Danish and NHANES populations that were detected in > 60% of the Danish population. Results are reported as volume-based (not creatinine- or lipid-adjusted). Table S1 in the Supplemental Files presents creatinine and lipid adjusted concentrations of chemicals presented in Table 3.

In general, concentrations of chemicals were of the same order of magnitude in the two populations, although geometric means and 95th percentiles tended to be higher in NHANES. Only blood mercury, urinary barium and enterolactone, a phytoestrogen, had higher concentrations in the Danish study population. The most pronounced contrasts between the two populations were for PAHs, PBDE153, benzophenone-3, and phthalate

metabolites, which had, on average, concentrations two, three, five, and two times higher, respectively, in NHANES.

Results of the PCA analysis are shown in Table 4, and component loadings are displayed in the Supplemental material, Tables S2A–S2C. To further identify commonalities between and within biomarker classes, we examined biomarker correlations within components. Below we discuss strong and moderate positive correlations. Heat maps displaying the correlations within each of the 7 components can be found in the Supplemental material, Figs. S1A–S1G.

Model 1 includes biomarkers measured in blood, serum and urine from 56 women. The proportion of variance explained by the three components described is 47.1%. Persistent pesticides, PCBs and total mercury loaded on Component 1. Within Component 1, PCBs had high intra-class correlation, ranging from 0.33 to 0.98. Omitting PCB74 and PCB105, which were not correlated with other PCBs, intra-class correlation ranged from 0.50 to 0.98. Both hexachlorobenzene and 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (pp′-DDE) were moderately correlated with PCBs (0.39–0.88). Although blood manganese and mercury loaded high on this component, they were not correlated with other biomarkers. PAHs, parabens, urinary metals and cotinine primarily comprised Component 2. We found that PAHs had high intra-class correlations (0.62–0.96), and were only highly correlated with perchlorate. PFAS biomarkers loaded separately on Components 2 and 3. Within Component 2, PFAS exhibited high intra- but not inter-class correlation. Phthalate metabolites, the primary chemical class loading on Component 3 of Model 1, also had high intra-class correlation, but only moderate correlations with PFAS and organophosphate metabolites. Of the 9 urinary trace and heavy metals detected in greater than 75% of the population, only cobalt and lead exhibited loadings greater than 0.25, the cutoff used for inclusion in any component. Phytoestrogens also did not load high on any components.

Among biomarkers measured only in urine, Model 2, two components explained 38.9% of the proportion total variance, and 11 components explained 100% of the variance (data not shown). In this model, PAHs, monoethyl phthalate and parabens loaded high on Component 1. Here, PAHs were moderately correlated with monoethyl phthalate. Arsenic and arsenic metabolites, all other phthalates and non-persistent pesticide metabolites largely explained Component 2. Here, total arsenic and arsenic metabolites had strong intraclass correlation and were moderately correlated with urinary strontium. Diethylphosphate, a nonspecific metabolite of OP pesticides, was also moderately correlated with 3,5,6-trichloro-2-pyridinol, a specific metabolite of the OP pesticide chlorpyrifos. With the exception of strontium and equol, all other urinary metals and phytoestrogens had component loadings less than 0.25.

For variables measured in blood and serum in Model 3, 39.3% of proportion of variance was explained by two components. Blood manganese, total mercury, PCBs and persistent pesticides loaded on Component 1. PFAS biomarkers, blood metals, cotinine and PCB28 explained Component 2. Biomarkers measured in blood and serum, exhibited fewer strong inter-class correlations than biomarkers measured in urine. The strongest correlations in this Model were PCBs 99, 238-158, 146, 153, 183 and 187 with pp′-DDE (Component 1). Blood metals had low intra- and inter-class correlation, while PFAS had high intra-class

correlation (Component 2). Cotinine and blood cadmium in Model 3, Component 2 were moderately correlated (0.58).

We conducted sensitivity analyses to assess the potential effects of pregnancy status, batch analyses, and detection frequency on our results. Four of the 73 women included in the study were in their first trimester of pregnancy when they enrolled. We compared descriptive statistics between pregnant and non-pregnant women, finding that values within chemical classes and spread of the data were generally the same between the two groups. We performed a batch analysis to compare concentrations between the first and second round of samples. Using bivariate regression, we examined the percent variability in biomarker concentration explained by an indicator variable for batch. We found that batch did not explain enough variability in concentration to warrant stratification by batch. Finally, we compared PCA results between the original data, the variance-covariance matrix of the imputed data, one of the imputed datasets and all of the imputed datasets. We found that loading patterns, and variance explained by the four factors, were similar across datasets.

4. Discussion

We found extensive exposure to persistent and non-persistent chemicals in this population of 73 Danish women of reproductive age. To our knowledge, no previous studies have measured over 130 different chemical biomarkers or analyzed correlations between a large number of exposure biomarkers in samples collected from the same person in a Danish population. With the ability to measure a wide array of known chemical biomarkers, we were further able employ PCA to examine correlations.

On average, 91 different chemicals were detected in the Danish women who participated in our study, with a minimum of 60 and a maximum of 108 of the 135 chemicals that were detectable. Chemical classes that were detected at the highest frequency (with all LODs being in the trace level range) included metals, metabolites of PAHs and phthalates, phytoestrogens and PFAS. Because few participants reported being occupationally exposed to the target chemicals, the results likely reflect general exposures among this group of Danish women. The generalizability of these results is further supported by recent large biomonitoring studies in reproductive-age Danish women that report similar biomarker concentrations of PCBs, PFAS biomarkers and metals to those reported in our study (de Renzy-Martin et al., 2014; Frederiksen et al., 2014; Jensen et al., 2015; Mørck et al., 2015b).

Biomarker concentrations were not markedly different between the SF/Milieu and NHANES populations, with the exception of PBDEs, benzophenone-3, PAHs and phthalate metabolites which were higher among NHANES women. All but three PBDEs measured in NHANES were detected in at least 60% of the population, while in Denmark only PBDE153 was commonly detected. Higher PAH and phthalate levels found in the NHANES population may be attributed to stricter regulations in Denmark, including a 2010 ban on the import, sale and use of PAHs in extender oils, and restriction of the use of PAHs in a wide range of consumer products in 2014 by the European Union. In 2012 the Danish Environmental Protection Agency also proposed a ban of phthalates in consumer and personal care products, bringing the potential reproductive effects of phthalate exposure into the public

eye (European Chemical Agency, 2015; The Danish Environmental Protection Agency, 2013). The United States only restricts phthalates in children's products and does not regulate PAHs in consumer products.

In addition to NHANES, several large biomonitoring programs have been conducted in European and North American countries over the last decade (Cerna et al., 2012; Den Hond et al., 2015; Fréry et al., 2012; Haines and Murray, 2012; Kolossa-Gehring et al., 2012; Mørck et al., 2015b; Perez-Gomez et al., 2013; Schoeters et al., 2012). Large-scale birth cohorts are also increasingly publishing biomonitoring results from their study populations (Guxens et al., 2012; Kawamoto et al., 2014). However, most of these studies only measure a few chemical classes or specific biomarkers simultaneously.

The Consortium to Perform Human Biomonitoring on a European Scale (COPHES) harmonized biomonitoring results of mercury, cadmium, cotinine and phthalate metabolites in mothers and children from 17 European countries. Geometric mean urinary cadmium concentration was lower in our study (0.167 μg/L) than the Danish study that contributed data to COPHES (0.219 μg/L), but higher than the full COPHES study (0.115 μg/L) (Mørck et al., 2015b; Smolders et al., 2015). Geometric mean blood cadmium and lead concentrations were slightly lower in our study population than among women participating in the BioMadrid Project and in the Canadian Health Measures Survey (CHMS) (García-esquinas et al., 2013; Haines and Murray, 2012). However, total mercury concentrations were higher in our study than in CHMS.

Non-persistent organic chemicals, such as phthalates, phenols, and parabens are synthetic chemicals used in a variety of consumer products. Participants in SF/Milieu had lower BPA concentrations (1.87 μg/L) than NHANES (2.79 μg/L), but higher concentrations than participants in the Swedish cohort contributing to COPHES (1.31 μg/L), and a separate Danish pregnancy cohort (1.17 μg/L) (de Renzy-Martin et al., 2014; K. Larsson et al., 2014). Triclosan was detected in less than 52% of our study population, which is contrary to the United States and other European studies that detect triclosan in greater than 70% of the population (Calafat et al., 2008; Frederiksen et al., 2014; de Renzy-Martin et al., 2014). Methyl paraben and propyl paraben concentrations were slightly lower in the current study than in the Danish COPHES study, and were two orders of magnitude lower compared to women in Spain and in France (Casas et al., 2011; Philippat et al., 2012). Phthalate metabolite concentrations were also lower in this study than in COPHES and Swedish women, but mono-isobutyl phthalate, monoethyl phthalate, and monobenzyl phthalate had higher concentrations than in another Danish pregnancy cohort (Den Hond et al., 2015; Frederiksen et al., 2014; S.C. Larsson et al., 2014). Phthalate metabolite concentrations in this population were also similar to a biomonitoring study of pregnant women in Peru (Irvin et al., 2010).

The major exposure pathway to pesticides is through residues commonly found on produce (Lu et al., 2006). However, ambient exposure to pesticides through air or dust may account for some variation in exposure (McKone et al., 2007). Notably, all classes of pesticides were detected at higher frequencies in NHANES than in Denmark. The wider concentration ranges among the NHANES participants than among the Danish study population may be

attributed to the large geographic variability of exposure or lifestyle characteristics in the United States. State-specific policies, around flame retardants for example, modulate individual exposure to select chemicals, whereas all participants in SF/Milieu were drawn from one city, Aalborg. Pesticides may have been more frequently detected among NHANES women because of the higher presence of pesticides in food in the United States as compared to Denmark. In Denmark, overall residues above the maximum residue limit were detected in only 2.6% of produce (Petersen et al., 2013), while a recent report by the U.S. Food and Drug Administration found that 21% of imported food contained residues that exceeded the Environmental Protection Agency tolerance residues (U.S. Food and Drug Administration, 2012). OP pesticides were also lower in our study than in women in the CHMS (Haines and Murray, 2012).

Persistent organic pollutants, including PCBs, BFRs, and organochlorine insecticides are lipophilic and distribute in fatty portions of tissues and breast milk in lactating women. They have a long half-life in the body and may distribute into fetal tissue (Barr et al., 2005). Despite banned use of organochlorine pesticides, they persist in the environment and may result in human exposure through diet. Flame retardants are not normally used in textiles produced in Denmark. The principal exposure to BFRs in the Danish population is likely through imported furniture, which explains PBDE153, a primary component of the pentaBDE mixture used in flame retardants, as the only detectable PBDE in the Danish participants (Lacasse and Baumann, 2004). Concentrations in the current study were lower than Danish mothers participating in a separate, small pregnancy cohort (Vested et al., 2014). PFAS concentrations were similar to the general Danish population but lower than concentrations found in the U.S. and other European countries (Mørck et al., 2015a). The discrepancy in Danish concentrations compared to other countries may be attributed to the voluntary phase out of PFAS chemicals by the industry beginning in 2000 (Mørck et al., 2015a).

Only a few other studies have evaluated correlations among a large number of exposure biomarkers (Agay-Shay et al., 2015; Patel and Ioannidis, 2014; Robinson et al., 2015). Agay-shay et al. (2015) performed PCA on 27 endocrine disrupting compounds in the INfanciay Medio Ambiente (INMA) birth cohort in Sabadell, Spain, as an approach to examine multi-pollutant exposure in association with child weight. Similar to our study, arsenic metabolites and PBDE153 loaded separately from their chemical classes, and persistent pesticides and phthalate metabolites had high intra-class correlation, suggesting similar exposure or metabolism patterns in these two populations. In contrast to our results, they found detectable levels of many PBDEs. Robinson et al. (2015) used PCA to explore correlation patterns between 81 chemical and non-chemical exposures, such as information about the home environment, the built environment, noise and surface temperature, in the same Spanish cohort. They found that correlations within chemical classes were greater than between classes.

Like the results in Robinson et al. (2015), higher within- than between-class correlations may reflect that chemical classes derive from disparate sources. Many of the component loadings reflect shared lifestyle patterns in this study population. The highly-loaded chemical biomarkers in this study are primarily found in consumer products and as industrial

byproducts. For instance, monoethyl phthalate, a breakdown product of diethyl phthalate and parabens loaded high on the same components. Both are commonly used in cosmetic products, such as hair and nail products, fragrances, make-up, and soaps, and in food packaging. Increasing levels of phthalates have been have been associated with both food and use in packaging (Mervish, 2014). PAHs and perchlorate were also found to be strongly correlated, which may be explained by the fact that they are both released by combustion processes. The correlations found between phthalates and PAH biomarkers with other chemicals classes may be attributed to their cooccurrence in indoor settings, where they have been extensively measured and detected in dust from Danish homes and internationally (Langer et al., 2010).

PFAS chemicals are primarily used as stain and grease repellants in cookware and furniture, and have been commonly detected in drinking water, with PFOS and PFOA being the most ubiquitous. Previous studies have found that PFAS are not correlated with other chemical classes, but exhibit high intra-class correlation, supporting the results of this study (Fisher et al., 2016; Gump et al., 2011; Mørck et al., 2015a). The observed high intra-class and low inter-class PFAS correlations explain Model 3, Component 2, where high exposure to one PFAS may be related to exposure to other PFAS biomarkers.

Cotinine is frequently used as a surrogate of cigarette consumption in epidemiological studies, and a positive association between levels of cadmium and smoking has been well established in both Danish populations and populations worldwide (Elinder et al., 1983; O'Connor et al., 1994; Richter et al., 2009). Here, we observed cadmium and cotinine loading on the same component, indicating that smoking status may contribute to a proportion of the variance explained by Component 2 in Models 1 and 3. In these two models, we also found that total mercury loaded with PCB congeners that are commonly found in food (138, 153, 180). In a study examining persistent chemical concentrations in the general Danish population, living in an urban area and consumption of saltwater fish were the most predictive factor of mercury in hair and PCB concentrations (Mørck et al., 2014). Saltwater fish consumption is higher in Denmark compared to other countries, which may directly explain PCBs and mercury loading on the same components in this study (Den Hond et al., 2015). The same study found that living in an urban area is also associated with higher pp′-DDE concentrations, which loaded with PCBs in both models.

We also observed that PCB 28 loaded separately from other PCB congeners. While most PCBs detected in this study population are typically found in food, PCB 28 is easily volatilized due to its low degree of chlorination and high vapor pressure. PCB 28 was primarily used in building sealants between the 1950s and 1970s, during which approximately 30% of the building stock in Denmark was constructed (Frederiksen et al., 2012; Kohler et al., 2002). We can therefore infer that PCB 28 loads separately from other PCBs due to exposure via inhalation versus ingestion from different exposure sources. Mørck et al. (2014) also found that PCB 28 displayed different exposure patterns than other PCB congeners in Danish women and children (Mørck et al., 2014). Together these findings suggest that observed correlations and factor loadings may be related to lifestyle patterns.

While most biomarkers with high detection frequencies explained enough variability in the data to load high on a component, neither urinary metals nor phytoestrogens appeared in the PCA components, despite their high detection frequencies. Low correlations between urinary metals and phytoestrogens with other chemical classes, explain their failure to load high on any of the components. These results suggest that metals and phytoestrogens are not derived from the same set of sources as biomarkers that explained the highest proportion of variation in the data. For instance, phytoestrogens are almost exclusively found the diet, specifically fruits, vegetables, soy products and cereal products. Metals are released into the environment from a diverse set of sources, including the natural geography and industry. The highly loaded chemical biomarkers in this study tend to be persistent, primarily found in consumer products, or as combustion byproducts.

A growing number of studies are using a multi-pollutant approach to relate exposures to health outcomes and refine exposure assumptions (Braun et al., 2014; Patel et al., 2010; Patel and Manrai, 2015). Several statistical and conceptual challenges exist in multi-pollutant epidemiological studies (DeBord et al., 2016). For instance, while some studies attempt to examine separate univariate associations with each predictive variable, the multitude of comparisons may result in type 1 errors. The univariate approach also assumes that each chemical acts independently, and overlooks potentially complex relationships within chemical mixtures (DeBord et al., 2016). Confounding by correlation and identifying valid confounders in multi-pollutant students can also challenge the ability to disentangle the true associations and result in erroneous conclusions.

Here, we used PCA to examine correlation patterns between 16 chemical classes. PCA is a useful method to address some of the methodological and conceptual challenges presented in mixture studies. The results of PCA summarize the data into latent constructs that explain variance in the data. If the patterns and correlations are clear enough, the outputted latent variables can be used as surrogates to the measured biomarkers. In statistical analysis, outputted component scored can be used as a method to avoid multi-pollutant confounding. PCA is also useful as a data visualization tool to understand the underlying data structure, which can be used for hypothesis generating and to highlight clusters of exposure (Pleil et al., 2011).

The results presented here can be used to identify shared exposure sources and as a means of focusing future epidemiology studies. Policymakers can also use these findings to focus regulation that addresses exposure patterns specific to this population. Further, these results add to the growing body of literature that monitor trends in environmental exposures, and can serve as an indicator of a shifting external environmental while trends change over time (Pleil et al., 2012).

4.1. Limitations

Our study has several limitations. Measurements taken in both the Denmark and NHANES populations were from single spot blood and urine samples taken at one point in time. For non-persistent chemicals, there is concern that spot urine measurements are subject to within-individual variability over time. Studies have found significant temporal variations of phthalate, phenol and organophosphate metabolites in urine samples (Bradman et al., 2007;

Cox et al., 2016; Engel et al., 2014; Meeker et al., 2013). Concentrations measured in this study likely provide imprecise estimates of long-term exposure (Calafat et al., 2015). In addition, some of the discrepancy in detected concentrations between this study and levels found in other countries may be attributed to higher limits of detection in the older NHANES rounds and in some of the older studies to which we compare our results.

We performed PCA with a small sample size and a high dimension, which has potential to yield unstable estimates. Previous studies have argued that good factor recovery may still be achieved despite small numbers (de Winter et al., 2009; Preacher and Maccallum, 2002). For instance, PCA has been used in genetic studies with small sample sizes (Reich et al., 2008).

Some authors have suggested that selection bias may be of concern in prospective cohort studies that employ internet-based recruitment (Keiding and Slama, 2015). Using Danish birth registry data, Hatch et al. (2016) found that six well-known perinatal associations were similar in the Snart Gravid study, a precursor to the SF study, and the general population. For example, the risk for low birth weight among women who smoked over 10 cigarettes in pregnancy compared with none was very similar in both populations (RR=2.7 in Snart Gravid and 2.9 in all of Denmark). Similar results in both populations were also found for maternal obesity and birth weight and parity in relation to risk of preeclampsia, suggesting that selection bias may not be a concern (Hatch et al., 2016). The four women in the current study who had positive pregnancy tests in their first visit may have biased descriptive statistics and PCA results. We performed sensitivity analysis to determine if concentrations between these pregnant and non-pregnant study participants was significantly different, but found no notable difference in biomarker concentrations between the two populations.

5. Conclusions

Studying the totality of environmental exposure is complex. By reporting on a wide array of chemical classes at once and identifying common co-exposures, the present study expands our understanding of the "pregnancy exposome," and underscores the importance of considering exposure to several chemical classes (Wang et al., 2016). We found widespread exposure to over 100 chemical biomarkers. Several chemical classes were highly correlated which may be indicative of common exposure sources, specific lifestyle factors or shared metabolic pathways (Patel and Manrai, 2015; de Renzy-Martin et al., 2014). Further studies using reported biomonitoring data and correlations can help identify shared sources and pathways for common exposures and may help elucidate the intricate chemical interactions that lead to disease pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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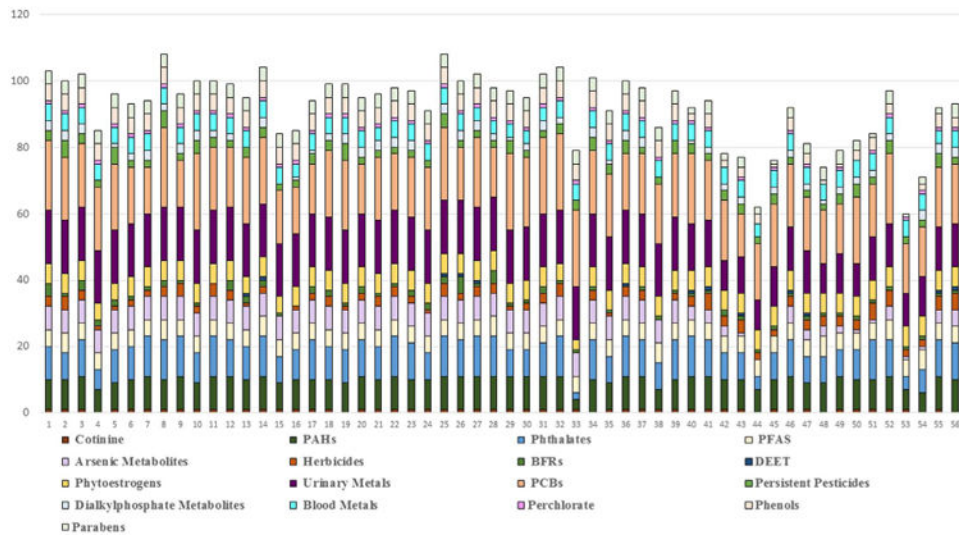
Fig. 1.
Number of biomarkers detected in each study participant (n=56). Each bar represents one individual. *Includes only those study participants with both urine and blood samples.

Table 1

Number of analytes measured in media and lab analytic methods.

Chemical Class	Blood	Serum	Urine	Total	Method	Ref.
Cotinine		1		1	HPLC-APCI Tandem Mass Spectrometry	(Centers for Disease Control CDC, 2008)
Metals	5		16	21	ICP-DRC-MS, HPLE- ICP-DRS-MS (total arsenic)	(Centers for Disease Control CDC 2012a, 2012b)
Arsenic Metabolites			7	7	HPLE-ICP-DRC-MS	(Centers for Disease Control CDC 2011a)
Polycylic Aromatic Hydrocarbons			10	10	GC-MS/MS	(Li et al., 2006)
Brominated Flame Retardants		11		11	GC-IDHRMS	(Jones et al. 2012)
Herbicides and Insecticides			8	8	RPHPLC and tandem mass spectrometry with isotope dilution quantitation	(Davis et al., 2013)
PCBs		24		24	GC-IDHRMS	(Jones et al., 2012)
Persistent Pesticides		8		8	GC-IDHRMS	(Jones et al., 2012)
Perchlorate			1	1	IC-MS/MS	(Centers for Disease Control CDC 2011b)
Phenols			5	5	HPLC-MS/MS	(Ye et al., 2005)
Parabens			4	4	HPLC-MS/MS	(Ye et al., 2005)
Phthalates			12	12	(HPLC)-electrospray ionization-tandem mass spectrometry	(Silva et al., 2007)
Phytoestrogens			6	6	LC-APPI-MS/MS NBB 4066	(Centers for Disease Control CDC, 2010)
Per- and polyfluoroalkyl substances		8		8	extraction–HPLC–isotope dilution–tandem mass spectrometry	(Kato et al., 2011)
Dialkylphosphate Metabolites			5	5	HPLC- electrospray ionization-tandem mass spectrometry	(Odetokun et al., 2010)
DEET Metabolites			4	4	HPLC-MS/MS	(Kuklenyik et al., 2013)
TOTAL				135		

Table 2

Characteristics of Snart Foraeldre/Milieu study population (n=73).

	n (%)
Education	
Basic Schooling (9 years)	3 (4.11)
Basic Schooling (> 9 years)	64 (87.67)
Other schooling	6 (8.22)
Additional Education	
None or Missing	2 (2.74)
Short, < 3 years (e.g. carpenter, electrician, nurse and dental assistants)	3 (4.11)
Medium, 3–4 years (e.g. teacher, RN nurse, B.A.)	39 (53.42)
Long, > 4 years (e.g. Master degree or Ph.D.)	29 (39.73)
Employment Status	
Work or apprenticeship	39 (53.42)
Unemployed or unemployed in job training	7 (9.59)
Student	25 (34.25)
Other	2 (2.74)
Household Income per Month	
Danish Krone < =39,999	35 (47.95)
Danish Krone >39,999	34 (46.58)
Refused or Don't Know	4 (5.48)
Parity	
0	51 (69.86)
1	17 (23.29)
2	5 (6.85)
Number Months Trying to Conceive	
Missing	1 (1.37)
0	14 (19.18)
1–3	33 (45.21)
4–7	15 (20.55)
8–12	5 (6.85)
> 12	5 (6.85)
Smoking	
Ever smoker	20 (27.40)
Never smoker	53 (72.60)
Daily Exposure to:	
Agricultural pesticides	1 (1.37)
Metal particulates or fumes	1 (1.37)
Solvents, oil-based paints, or cleaning compounds	5 (6.85)
Environments with temperatures above 25°	3 (4.11)
Engine exhaust	19 (26.03)
	Mean ± SD

	n (%)
Age	28.1 ± 4.3
BMI	23.9 ± 4.6

Table 3

Chemical Analyte Descriptive Statistics in Snart Foraeldre/Milieu Population and NHANES Population.[a,b]

	Snart Foraeldre/Milieu				NHANES[c]					
	N	GM	10th Pctl	95th Pctl	% >LOD	N[c]	GM	10th Pctl	95th Pctl	% >LOD	NHANES Year
Cotinine (ng/mL)											
Cotinine	73	0.09	0.01	120	86	1216	36.58	0.011	258	100	11–12
Metals_Blood (ng/mL except lead which is µg/dL)											
Blood cadmium	73	0.2	0.071	0.76	88	1234	0.46	0.11	1.57	84.12	11–12
Blood manganese	73	10.52	7.66	17.65	100	1234	10.95	6.84	18.69	100	11–12
Blood lead	73	0.81	0.53	1.58	100	1234	0.72	0.32	1.54	97.57	11–12
Blood selenium	73	115.02	98.52	140	100	1234	192.0	162.88	234.96	100	11–12
Blood mercury, total	73	1.59	0.69	5.2	97	1234	1.14	0.21	3.79	96.84	11–12
Metals (ng/mL)											
Barium	50	0.68	0.17	2.7	100	410	1.57	0.29	4.34	98.54	11–12
Cadmium	50	0.1	0.03	0.52	80	410	0.19	0.04	0.63	80.73	11–12
Cobalt	50	0.28	0.07	1.06	100	410	0.55	0.10	1.58	98.78	11–12
Cesium	50	2.97	0.95	10.6	100	410	4.39	1.13	10.8	100	11–12
Molybdenum	50	25.98	5.54	134	100	410	47.01	9.04	121	100	11–12
Lead	50	0.24	0.08	0.88	98	410	0.35	0.06	1.58	91.22	11–12
Antimony	50	0.04	0.02	0.15	72	410	0.06	0.03	0.15	54.39	11–12
Tin	46	0.16	0.06	0.63	65	410	1.00	0.16	4.04	79.02	11–12
Strontium	50	79.69	22.06	333.39	100	410	107.94	20.04	282.98	100	11–12
Thallium	50	0.15	0.04	0.46	100	410	0.18	0.05	0.44	99.27	11–12
Tungsten	50	0.05	0.01	0.28	78	409	0.18	0.02	0.44	80.44	11–12
Uranium	50	0	0	0.02	68	410	0.01	0.002	0.02	64.88	11–12
Arsenic, total	57	9.25	1.9	48.34	100	410	14.77	1.83	54.90	94.15	11–12
Arsenic Metabolites (ng/mL)											
Arsenobetaine	57	5.51	0.82	53.8	78	412	8.49	0.84	36.90	49.03	11–12
Dimethylarsonic acid	57	2.93	1.35	7.46	69	412	4.53	1.27	11.40	76.7	11–12
Monomethylarsonic acid	57	0.43	0.14	1.64	69	412	0.79	0.63	1.51	24.03	11–12

	Snart Foraeldre/Milieu				NHANES[c]					
	N	GM	10th Pctl	95th Pctl	% >LOD	N[c]	GM	10th Pctl	95th Pctl	% >LOD	NHANES Year
PAH Biomarkers (ng/L)										
1-hydroxynaphthalene	56	309.32	72.81	5907.16	98	410	3806.53	248	19,250	100	11–12
1-hydroxyphenanthrene	56	67.92	23.26	589.83	98	410	178.78	36	540	99.76	11–12
1-hydroxypyrene	56	58.96	20.00	458.60	98	410	212.64	34	671	99.27	11–12
2-hydroxyfluorene	56	95.32	24.68	865.00	100	410	512.62	49	2394	100	11–12
2-hydroxynaphthalene	56	1158.68	237.54	15,869.08	100	410	9464.10	854	30,078	100	11–12
2-hydroxyphenanthrene	56	30.94	7.07	230.47	88	410	86.93	17	246	96.83	11–12
3-hydroxyfluorene	56	39.49	10.61	499.19	91	410	244.84	16	1257	97.07	11–12
3-hydroxyphenanthrene	56	30.68	7.07	278.56	84	410	91.71	15	295	94.39	11–12
4-hydroxyphenanthrene	56	13.73	7.07	99.37	46	409	30.17	7.1	96	75.79	11–12
9-hydroxyfluorene	56	113.1	28.81	1178.22	100	410	409.88	47	1216	100	11–12
Brominated Flame Retardants (pg/g)										
PBDE153	73	6.43	3.6	14.2	95	363	66.47	8.6	319.1	92.56	03–04
PCBs (ng/g)										
PCB99	73	0.02	0.01	0.04	99	326	0.02	0.01	0.06	100	03–04
PCB138 & 158	73	0.11	0.05	0.28	100	332	0.07	0.02	0.18	100	03–04
PCB146	73	0.02	0.01	0.06	99	331	0.01	0	0.02	98.49	03–04
PCB153	73	0.16	0.07	0.39	100	331	0.08	0.02	0.22	100	03–04
PCB170	73	0.04	0.02	0.12	99	332	0.02	0.01	0.05	97.69	03–04
PCB178	73	0.01	0	0.03	82	331	0	0	0.01	71.3	03–04
PCB180	73	0.10	0.04	0.28	100	332	0.06	0.01	0.16	100	03–04
PCB183	73	0.01	0.01	0.03	96	332	0.01	0	0.02	87.95	03–04
PCB187	73	0.03	0.01	0.09	100	332	0.02	0	0.04	98.8	03–04
PCB194	73	0.01	0.01	0.04	25	322	0.01	0	0.04	78.26	03–04
PCB199	73	0.01	0.00	0.04	86	330	0.01	0	0.03	85.76	03–04
Persistent Pesticides (ng/g)										
Hexachlorobenzene	73	0.06	0.04	0.1	100	36	0.05	0.04	0.07	100	07–08
p,p′-DDE	73	0.18	0.09	0.62	99	36	1.14	0.41	2.63	100	07–08
Perchlorate (ng/mL)										

	Snart Foraeldre/Milieu					NHANES[c]					
	N	GM	10th Pctl	95th Pctl	% >LOD	N[c]	GM	10th Pctl	95th Pctl	% >LOD	NHANES Year
Perchlorate	56	2.14	0.53	18.9	100	401	3.56	0.75	11.1	100	11–12
Phenols (ng/mL)											
Butyl Paraben	56	0.29	0.53	1.58	63	409	3.31	0.14	16.2	46.21	11–12
benzophenone-3	56	3.64	0.14	211	86	409	462.61	10.4	2230	99.76	11–12
bisphenol A	56	0.99	0.2	6.7	95	409	2.91	0.28	10.60	88.51	11–12
Methyl Paraben	56	18.16	2.2	475	96	409	264.89	7.30	1120.0	100	11–12
Propyl Paraben	56	1.67	0.07	228	86	409	80.65	1.20	309.0	99.27	11–12
Phthalates (ng/mL)											
Mono-n-butyl phthalate	56	5.41	1.1	37.2	95	409	19.37	0.5	46.8	91.69	11–12
Mono-benzyl phthalate	56	1.37	0.21	10.2	82	409	10.11	0.50	37.3	97.56	11–12
Mono(carboxynonyl) phthalate	56	0.37	0.14	1.8	66	409	3.83	0.7	11.4	98.53	11–12
Mono(carboxyoctyl) phthalate	56	2.3	0.5	20.2	96	409	59.67	4	248.9	100	11–12
Mono-(3-carboxypropyl) phthalate	56	0.47	0.14	2.6	63	409	9.31	0.5	30.0	97.8	11–12
Mono-2-ethyl-5-carboxypentyl phthalate	56	4.72	1.1	31.6	98	409	25.08	3.1	76.8	99.76	11–12
Mono-2-ethyl-5-hydroxyhexyl phthalate	56	2.83	0.8	14.7	96	409	15.99	1.1	49.5	99.76	11–12
Mono-2-ethylhexyl phthalate	56	0.75	0.35	4.1	95	409	3.87	0.35	9.7	75.06	11–12
Mono-2-ethyl-5-oxohexyl phthalate	56	1.91	0.4	11.5	95	409	10.58	1.2	31.9	99.27	11–12
Monoethyl phthalate	56	12.53	2.1	163	98	409	138.24	12.5	388.0	99.76	11–12
Mono-isobutyl phthalate	56	11.72	1.8	138	100	409	11.71	1.2	35.1	98.53	11–12
Phytoestrogens (ng/mL)											
Daidzein	56	34.75	2.6	791	98	497	462.34	6.5	1980	100	09–10
O-Desmethylangolensin	56	0.99	0.14	35.3	88	497	121.86	0.3	597	94.16	09–10
Equol	56	15.87	3.01	194	100	497	115.99	2.84	81.3	100	09–10
Enterodiol	56	82.11	8.2	1440	100	497	157.17	5.17	553	99.4	09–10
Enterolactone	56	836.79	141	8360	100	497	669.63	16.3	2610	100	09–10
Genistein	56	6.96	0.5	245	98	497	217.07	3.2	545	100	09–10
Diakylphosphate Metabolites (ng/mL)											
Diethylphosphate	55	0.55	0.07	5.67	75	425	3.53	3.53	14.7	31.06	07–08
Dimethylthiophosphate	56	0.5	0.07	4.97	77	426	7.26	7.26	36.8	73.94	07–08

	Smart Foraeldre/Milieu				NHANES[c]					
	N	GM	10th Pctl	95th Pctl	% >LOD	N[c]	GM	10th Pctl	95th Pctl	% >LOD	NHANES Year
Organophosphates (ng/mL)											
3,5,6-trichloropyridinol	56	0.66	0.07	5.59	88	482	1.34	0.07	4.02	80.71	09–10
Para–nitrophenol	55	0.32	0.07	2.86	78	482	0.79	0.07	2.65	78.01	09–10
PFAS (ng/mL)											
Perfluorodecanoic acid	73	0.27	0.2	0.5	100	375	0.24	0.07	0.64	80	11–12
Perfluorohexane sulfonic acid	73	0.31	0.2	0.7	100	375	1.21	0.33	4.28	97.87	11–12
Perfluorononanoic acid	73	0.58	0.3	1.1	100	375	0.93	0.37	2.07	99.73	11–12
Perfluorooctanoic acid	73	1.55	0.7	3.9	100	375	1.88	0.75	3.15	100	11–12
Perfluorooctane sulfonic acid	73	5.55	2.6	11.7	100	375	5.36	1.76	12.50	0	11–12

[a] Biomarkers chosen for inclusion and if detected in over 60% of the population.

[b] Results presented in volumetric concentrations.

[c] The number of NHANES women included in analysis varied because of missing data, whether the biomarker was measured in a pooled subsample of the NHANES population and by sampling year. As a result, between 36 and 1234 women were included to obtain the NHANES concentrations for each biomarker. In the Danish study population, the number of women included in descriptive statistics analyses varied due to missing data.

Table 4

Summary of correlations among 135 analytes measured in urine and blood among Snart Foraeldre/Milieu women (n=56). Includes only biomarkers detected in greater than 75% of the study population.[a]

	Component 1	Component 2	Component 3
Model 1: Blood and Urine	**Blood Metals**: manganese, Total Hg	**Blood Metals**: cadmium, lead	**PFAS**: PFDeA, PFNA, PFOA
		Urinary Metals: cobalt, lead	**PCB28**
		PFAS: PFHxS, PFOS	**PBDE153**
		Cotinine	**PAHs:** 1-hydroxyphenanthrene
	Persistent Pesticides: hexachlorobenzene, pp′-DDE	**PAHs**	**Urinary Metals**: total arsenic, arsenobetaine
	PCBs	**Perchlorate**	**Phthalates**
		Phthalate: Monoethyl phthalate **Parabens**: methyl-paraben, propyl-paraben	**Pesticides**: para-Nitrophenol, 3,5,6-Trichloro-2-pyridinol, diethylphosphate
Proportion Variance Explained	23%	16%	8.1%
Model 2: Urine Only	**PAHs**	**Arsenic and Arsenic Metabolites**	N/A
	Phthalate: Monoethyl phthalate	**Phthalates**	
	Parabens: methyl-paraben, propyl-paraben	**Pesticides**: para-Nitrophenol, 3,5,6-Trichloro-2-pyridinol, diethylphosphate	
	Phytoestrogen: Equol		
	Urinary Metals: lead	**Urinary Metals**: strontium	
Proportion Variance Explained	24%	14.9%	
Model 3: Serum and Whole Blood Only	**Blood Metals**: manganese, Total Hg	**All PFAS biomarkers**	N/A
	Persistent Pesticides: hexachlorobenzene, pp′-DDE	**Blood Metals**: cadmium, lead	
	PCBs	**Cotinine**	
	PBDE153	**PCB28**	
Proportion Variance Explained	24%	15.3%	

[a]Biomarkers with loadings < 0.25 are not listed.