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Identification of chemical mixtures to which Canadian pregnant women are exposed: The MIREC Study

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ABSTRACT

Depending on the chemical and the outcome, prenatal exposures to environmental chemicals can lead to adverse effects on the pregnancy and child development, especially if exposure occurs during early gestation. Instead of focusing on prenatal exposure to individual chemicals, more studies have taken into account that humans are exposed to multiple environmental chemicals on a daily basis. The objectives of this analysis were to identify the pattern of chemical mixtures to which women are exposed and to characterize women with elevated exposures to various mixtures. Statistical techniques were applied to 28 chemicals measured simultaneously in the first trimester and socio-demographic factors of 1744 participants from the Maternal-Infant Research on Environment Chemicals (MIREC) Study. Cluster analysis was implemented to categorize participants based on their socio-demographic characteristics, while principal component analysis (PCA) was used to extract the chemicals with similar patterns and to reduce the dimension of the dataset. Next, hypothesis testing determined if the mean converted concentrations of chemical substances differed significantly among women with different socio-demographic backgrounds as well as among clusters. Cluster analysis identified six main socio-demographic clusters. Eleven components, which explained approximately 70% of the variance in the data, were retained in the PCA. Persistent organic pollutants (PCB118, PCB138, PCB153, PCB180, OXYCHLOR and TRANSNONA) and phthalates (MEOHP, MEHHP and MEHP) dominated the first and second components, respectively, and the first two components explained 25.8% of the source variation. Prenatal exposure to persistent organic pollutants (first component) were positively associated with women who have lower education or higher income, were born in Canada, have BMI ≥25, or were expecting their first child in our study population. MEOHP, MEHHP and MEHP, dominating the second component, were detected in at least 98% of 1744 participants in our cohort study; however, no particular group of pregnant women was identified to be highly exposed to phthalates. While widely recognized as important to studying potential health effects, identifying the mixture of chemicals to which various segments of the population are exposed has been problematic. We present an approach using factor analysis through principal component method and cluster analysis as an attempt to determine the pregnancy exposome. Future studies should focus on how to include these matrices in examining the health effects of prenatal exposure to chemical mixtures in pregnant women and their children.

1. Introduction

Exposures to environmental chemicals during early life, either in utero or during early stages of childhood development, can impact fetal development and child health and may even lead to or exacerbate chronic conditions (Gluckman and Hanson, 2004). The rising rates of health problems such as infertility, autism, attention deficit and hyperactivity disorders, childhood brain cancer and acute lymphocytic leukemia, all thought to be associated with multiple causal factors, have further increased the interest in studying chemical mixtures (Bellinger, 2012). Studies have reported associations between several individual chemicals (e.g., pesticides, bisphenol A (BPA), phthalates, polybrominated diphenyl ethers (PBDEs) and heavy metals) and child neurodevelopment outcomes (Bellinger, 2012). Furthermore, other research suggests that many chemicals have similar mechanisms of action (e.g., endocrine disrupting effects) (Crofton et al., 2005; Kjeldsen et al., 2013) and exposure to multiple chemicals might have more than additive effects (National Research Council, 2008; Woodruff et al., 2011). This concept of the "exposome", defined as the totality of human environmental exposures from conception onward, complementing the genome, has attracted growing interest in recent years (Robinson et al., 2015). Varshavsky et al. (2016) used National Health and Nutrition

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Examination Survey data (NHANES, 2001–2012) and developed a potency-weighted sum of daily intake to examine demographic differences in cumulative phthalates exposure among U.S. women of reproductive age. Braun et al. (2016) point out that the health effects of cumulative exposure to multiple agents is one of the major questions in ongoing epidemiological studies.

Although the importance of chemical mixtures has been recognized for some time, rigorous study of their levels and impact has been slow due to a lack of knowledge, analytical capacity and funding (Lokke et al., 2013). This difficulty in understanding and predicting the effects of multiple exposures has been described as one of the greatest limitations in risk assessment (NAS, 2012). Little is known about the extent or impact of such multiple exposures in pregnant women. One possible explanation for this lack of knowledge is that, due to the large number of variables with potential impacts, the results of traditional statistical analyses, such as multiple linear models considering interaction between covariates, are sometimes difficult to interpret. However, statistical approaches exist which examine mixtures of chemicals accounting for much of the observed differences in exposure data. For example, where data sets have high dimensions (i.e. many variables) or high collinearity (i.e. highly correlated explanatory variables), a technique known as principal component analysis (PCA) is often used to reduce the dimension of the data and convert the raw data into linearly independent factor scores (Johnson and Wichern, 2007). PCA has been applied in risk assessment (Agay-Shay et al. 2015; Robinson et al. 2015; Veyhe et al. 2015). Another technique, called cluster analysis, can be used to assess similarities among subjects, such as similarities based on socio-demographic information. Such clusters could then be treated as independent variables for further association analysis between chemical mixtures and markers of disease risk or health outcomes. For example, nutritionists have incorporated cluster analysis to evaluate dietary patterns which reflect combinations of foods (i.e. mixtures) to identify individuals who may be at risk for certain health outcomes (Bailey et al., 2006; Funtikova et al., 2015; Clarke et al., 2015). Cluster analysis is also common in environmental science studies (Lampa et al., 2012; Lalloué et al., 2015; Nordio et al., 2015; OBrien et al., 2014; Peng et al., 2016; Zhao et al., 2016). Lampa et al. (2012) applied cluster analysis to the NHANES 2003–2004 and the Vasculature in Uppsala Seniors (PIVUS) studies, respectively, to assess possible clustering of environmental chemical contaminants (37 chemicals from PIVUS and 18 from NHANES) and the results showed some stable clusters. Lalloué et al. (2015) collected 31 environmental indicators from the Great Lyon area in France at the census Block Group (BG) scale. Cluster analysis was used to assess the environmental burden experienced by populations and five BG classes were categorized. Nordio et al. (2015) used cluster analysis to group the 211 cities in the US that share common weather characteristics. In order to evaluate air pollution situations in major cities in China, Zhao et al. (2016) measured pollutants $PM_{2.5}$, PM_{10}, SO_2, No_2, CO and O_3 between 2014 and 2015 from 31 provincial capital cities. Cluster analysis was used to understand the pollution levels among cities. For each pollutant ($PM_{2.5}$, PM_{10}, SO_2, No_2, CO and O_3) data were collected from multiple time points and sites in each of the 31 cities. Subsequently, the cities were then grouped according to similar air pollution levels.

Traditional statistical methods have been utilized in environmental health in recent years but these advanced methods can only be used when their statistical assumptions are satisfied. Data-driven approaches would be proposed when the assumptions are violated. Cluster analysis using a Bayesian nonparametric approach and PCA were applied to estimates of dietary pesticide levels to identify the main mixture of pesticides to which the general population is exposed in France (Crépet et al., 2013). The same dataset was also analyzed by the method of Nonnegative Matrix Factorization, which basically decomposed the matrix of individuals' consumption quantities; and PCA was used to examine the main mixture to which the French population was exposed and the connection between exposure and diet (Béchaux et al., 2013). Herring (2010) examined the association between endometriosis and exposure to environmental polychlorinated biphenyl (PCB) congeners by multiple logistic regression considering Bayes shrinkage priors. Sun et al. (2013) summarize five statistical methods (classification and regression tree, supervised principal component analysis, least absolute shrinkage and selection operator, partial least-squares regression, Bayesian model averaging) for constructing multipollutant models and conduct a simulation study to assess the performance of these five models. Bobb et al. (2014) introduced Bayesian kernel machine regression to study mixture in which the health outcome is regressed on a high-dimensional exposure-response function of the chemical mixtures that is specified using a kernel representation. However, as these approaches are data-driven, the chemical mixtures developed using these methods may not always lead to results which are easy to interpret.

The Maternal-Infant Research on Environment Chemicals (MIREC) Study was developed to investigate the impacts of environmental chemicals on the health of pregnant women and their offspring and to identify vulnerable (exposed) subgroups within the population (Arbuckle et al., 2013). The one-chemical-at-a-time approach provides insufficient knowledge about the human health effects of exposure to chemical mixtures (Braun et al., 2016). In this study, we developed statistical criteria to examine the association between exposure to chemical mixtures and maternal socio-demographic characteristics. Our objectives were to (Agay-Shay et al., 2015) apply cluster analysis to identify sub-groups of pregnant women by their socio-demographic characteristics; (Ashley-Martin et al., 2015) apply PCA to first-trimester environmental chemical concentrations in blood and urine of pregnant women to search for patterns among the contaminants that are potentially highly correlated; and (Arbuckle et al., 2014) utilize these components together with cluster analysis results and hypothesis testing to identify the socio-demographic characteristics of pregnant women with high exposures to multiple chemicals. While many statistical approaches are available, we focused on commonly used techniques in an effort to produce interpretable results.

2. Methods

2.1. Study population and data collection

The MIREC pregnancy cohort study has been described previously (Arbuckle et al., 2013). Briefly, approximately 2000 pregnant women were recruited in early pregnancy (<14 weeks) from prenatal clinics in ten cities across Canada between 2008 and 2011 and followed over the course of pregnancy and infant birth. Participants completed a detailed questionnaire covering socio-demographic details from which information on age, education, household income, parity, pre-pregnancy body mass index (BMI), country of birth and smoking status was extracted. The protocol for the MIREC Study was reviewed by multiple research ethics committees and all study participants signed informed consent forms.

Blood and urine samples were collected during the 1st trimester of pregnancy for chemical analyses. Chemicals considered in these analyses included metals (arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), manganese (Mn)), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), and perfluoroalkyl substances (PFASs) measured in blood, as well as bisphenol A (BPA), organophosphate pesticides (OPs) and phthalate metabolites measured in urine.

2.2. Statistical analysis

To account for all seven socio-demographic variables (age, education, household income, parity, pre-pregnancy body mass index (BMI), country of birth and smoking status) simultaneously, we first performed a cluster analysis to categorize the pregnant women. As demographic variables were either discrete or continuous, the Gower

distance was chosen to measure the similarities between subjects. The *diana* algorithm in software R (a divisive hierarchical clustering of the dataset) was used to perform the cluster analysis.

In order to maintain statistical reliability, chemicals with less than approximately 30% of samples below the limit of detection (LOD) were omitted from further analysis. For the remaining chemicals, values below the LOD were substituted by one half the limit of detection. Standardization was applied to convert the raw data into values without the unit of measurement, a step recommended for using PCA when the variance of the variables are heterogeneous (Johnson and Wichern, 2007). Through PCA, we converted our raw data into independent factor scores based on factor loadings to examine the association between the factor scores and characteristics of the pregnant women. To illustrate the PC (principal component) scores, suppose the vector $(x_1, x_2, \cdots, x_{28})$ records the chemical concentrations of Mn, Pb, ···, beta-Hexachlorocyclohexane (B-HCH) for a single participant. The following equation

$$0.019x_1 + 0.1611x_2 + \cdots + 0.1054x_{28}$$

was then used to convert the chemical concentrations into a PC1 score for each subject. Each score is derived from this linear combination of the measured chemical concentrations. As demonstrated in the Results section, since the values corresponding to PCB118, PCB138, PCB153, PCB180, oxychlordane (OXYCHLOR) and trans-nonachlor (TRANSNONA) (Table 5) are positive and higher than those seen for the other 22 chemicals, higher concentrations of these chemical substances would lead to higher PC1 scores. Similarly for the second component (PC2), the linear equation

$$-0.0317x_1 + (-0.02)x_2 + \cdots + (-0.0002)x_{28}$$

was used to determine a PC2 score for each subject. Since the eigenvalues of PC2 corresponding to mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-2-ethylhexyl phthalate (MEHP) are negative and smaller than those of other chemicals, higher concentrations of these chemical substances would lead to smaller PC2 scores. Components were retained for further analysis if a component had an eigenvalue of at least one or at least 70% of the source variation was explained by the retained components.

Then we examined the association between the factor scores (the response variables) and the pregnant women in terms of the socio-demographic characteristics and the clusters (the covariates). Continuous covariates were analyzed using linear regression, while ANOVA was applied to test for the association for discrete covariates. The aim of ANOVA was to determine whether there were significant differences among mean factor scores in terms of the characteristics of the participants and the clusters. If the ANOVA test was statistically significant, Tukey's honestly significant difference (HSD) test for multiple comparisons was then applied to test whether the pairwise differences of the mean scores were significantly different from zero. Regarding a continuous covariate, we fitted a linear regression model of the factor scores on maternal age and tested if the slope was significantly different from zero. The statistical analysis was performed using the R package version 3.1.1, and a significance level of 5% was assumed throughout.

3. Results

Concentrations of 28 chemicals out of 81 available chemicals were measured in the blood and urine samples from 1744 women. Table 1 summarizes descriptive statistics for the chemicals or their metabolites under study. These chemicals were found at detectable levels in approximately 70% of subjects, with lead (Pb) and manganese (Mn) detected in 100% of the women. Descriptive statistics for the 53 chemicals with higher percentages of non-detects are provided in the Supplemental material Table S1. Table 2 presents frequency distributions of the demographic variables for the 1744 MIREC participants. Maternal age ranged from 18 to 48 years, with a median age of 32 years. Most women were in their first or second pregnancy, had completed post-secondary education, had high income and were born in Canada. Almost 6% of the participants were current smokers, while another 6% had quit smoking during pregnancy. Fig. 1 presents a heat map of the Pearson correlation matrix of the 28 chemicals. Note that the chemical names in the x- and y-axes are colored according to their class and the chemicals inside each rectangle are the ones that dominated the component (Table 5).

3.1. Extreme values

When evaluating the chemical mixtures, some women were found to have extremely high levels of one or more chemicals. We identified a data point as an extreme value ("high level") if it was 100 times its interquartile range above the third quartile (if the threshold determined from this equation is <10, then 10 is used to identify an extreme value). Among the 1320 participants who were born in Canada, 3.17% had extreme values, while among the 324 participants who were born outside Canada, 6.79% had extreme values. Among 1479 pregnant women who were in their first or second pregnancy, 61 (4.12%) had extreme values; while among the 265 pregnant women who already had more than one child, only six (2.26%) had extreme values. Among the 105 pregnant women who quit smoking during pregnancy, eight (7.62%) had extreme values, while among those pregnant women who were non-smokers (n = 1063) and former smokers (n = 472), 40 and 8 (3.76% and 3.81%) had extreme values. Among 1744 subjects 55 women had one extreme high chemical level, 5 had two extreme high chemical levels and 5 had three extreme high chemicals levels. As a percentage, 3.73% (=65/1744) of pregnant women had at least one extremely high chemical level while, among women with extreme values, 15.38% (=10/65) had more than one extreme chemical level.

3.2. Cluster analysis

The cluster analysis as shown in Tables 3 and 4 resulted in six clusters of the 1744 participants. Cluster 1 included women born in Canada with a high income and high education level; Cluster 2 included women born outside of Canada and with a pre-pregnancy BMI lower than 25; Cluster 3 included women born in Canada with a middle income level; Cluster 4 included women who were born outside of Canada and with a pre-pregnancy BMI at least 25; Cluster 5 included women born in Canada with a low income level; and, Cluster 6 included women born in Canada with a high income level and low education level.

3.3. PCA analysis

We retained eleven components (PC1–11), which explained approximately 70% of the source variation. Table 5 shows the eigenvectors of the corresponding 11 components after rotation. The first component (PC1) accounted for 15.03% of the source variance and is dominated by PCBs and other persistent organic pollutants (POPs).

3.4. ANOVA and regression

Table 6 provides results from the ANOVA and linear regression analysis and the corresponding p-values for hypothesis testing. For example, the PC1 scores appear to be heavily influenced by the level of education (p-value < 0.001), which indicates that at least one pair of PC1 mean scores among the education levels are significantly different. With the exception of PC2, most demographic factors are significant in terms of their mean PC scores (Table 6). We, therefore, performed Tukey's HSD *post-hoc* tests to determine the differences among groups. The slope of the regression model of PC1 on maternal age is significant, which means maternal age is a good predictor for PC1 score.

Table 1
Descriptive statistics and percentage of non-detectable values for chemical concentrations in the first trimester samples from the MIREC Study (n = 1744) for chemicals with approximately 70% detectable observations.

Abbreviation	Description	Matrix	Units	% <LOD	MIN	Q1	Median	Q3	Max	Mean	STD	GE
Metals												
Mn	Manganese	Blood	nmol/L	0.00%	37.00	130.00	160.00	200.00	530.00	168.34	54.82	160.13
Pb	Lead	Blood	μmol/L	0.00%	0.01	0.02	0.03	0.04	0.25	0.03	0.02	0.03
Cd	Cadmium	Blood	nmol/L	2.63%	0.20	1.20	1.80	2.80	50.00	2.94	4.11	1.93
As	Arsenic	Blood	nmol/L	7.48%	1.50	6.97	11.00	16.00	460.00	13.49	17.88	9.86
Hg	Mercury	Blood	nmol/L	9.49%	0.25	1.60	3.50	6.80	50.00	5.04	5.19	3.04
DMAA	Dimethylarsinic acid	Urine	μmol/L	14.12%	0.01	0.02	0.03	0.06	0.86	0.05	0.07	0.03
Plasticisers												
BPA	Bisphenol A	Urine	μg/L	12.29%	0.10	0.34	0.77	1.60	140.00	2.03	7.47	0.76
MEP	Mono ethyl phthalate	Urine	μg/L	0.11%	0.25	11.00	28.00	86.00	13,000.00	137.25	511.70	31.85
MCPP	Mono-3-carboxypropyl phthalate	Urine	μg/L	15.22%	0.10	0.31	0.93	2.10	100.00	2.61	6.83	0.87
MnBP	Mono-n-butyl phthalate	Urine	μg/L	0.22%	0.10	5.20	12.00	25.00	3100.00	28.07	113.33	11.62
MEOHP	Mono-(2-ethyl-5-oxohexyl) phthalate	Urine	μg/L	0.28%	0.10	3.00	6.50	13.00	980.00	15.16	47.96	6.40
MBzP	Mono benzyl phthalate	Urine	μg/L	0.50%	0.10	2.30	5.20	12.00	420.00	12.19	25.39	5.22
MEHHP	Mono-(2-ethyl-5-hydroxyhexyl) phthalate	Urine	μg/L	0.62%	0.20	4.10	9.40	20.00	1200.00	23.52	74.10	9.18
MEHP	Mono-2-ethylhexyl phthalate	Urine	μg/L	1.52%	0.10	1.10	2.20	4.50	340.00	5.74	19.80	2.29
Perfluoroalkyl substances (PFASs)												
PFOA	Perfluorooctanoic acid	Plasma	μg/L	0.15%	0.05	1.10	1.70	2.40	16.00	1.95	1.24	1.65
PFOS	Perfluorooctane sulfonate	Plasma	μg/L	0.15%	0.15	3.30	4.60	6.70	36.00	5.29	3.07	4.54
PFHxS	Perfluorohexane sulfonate	Plasma	μg/L	4.12%	0.10	0.66	1.00	1.60	40.00	1.46	1.88	1.02
PCBs												
PCB118	2,3′,4,4′,5-Pentachlorobiphenyl	Plasma	μg/L	26.61%	0.01	0.01	0.01	0.02	0.22	0.02	0.02	0.01
PCB138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	Plasma	μg/L	7.03%	0.01	0.02	0.03	0.04	0.43	0.03	0.03	0.03
PCB153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	Plasma	μg/L	1.29%	0.01	0.03	0.04	0.07	0.93	0.06	0.07	0.04
PCB180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	Plasma	μg/L	7.39%	0.01	0.02	0.03	0.05	1.10	0.04	0.06	0.03
Organophosphate pesticides (OPs)						0.00	0.00					
DMTP	Dimethylthiophosphate	Urine	μg/L	19.92%	0.30	0.84	2.90	8.20	210.00	8.30	16.37	2.73
DMP	Dimethylphosphate	Urine	μg/L	20.83%	0.50	1.20	2.70	6.00	190.00	5.26	8.72	2.62
DEP	Diethylphosphate	Urine	μg/L	22.83%	0.50	1.00	2.10	4.20	3400.00	5.75	81.61	2.06
Organochlorine pesticides (OCs)						0.00	0.00					
DDE	p,p′-Dichlorodiphenyldichloroethylene	Plasma	μg/L	1.03%	0.05	0.20	0.30	0.48	26.00	0.58	1.34	0.34
OXYCHLOR	Oxychlordane	Plasma	μg/L	7.81%	0.00	0.01	0.01	0.02	0.10	0.01	0.01	0.01
TRANSNONA	Trans-nonachlor	Plasma	μg/L	15.87%	0.01	0.01	0.02	0.03	0.23	0.02	0.02	0.02
B-HCH	beta-Hexachlorocyclohexane	Plasma	μg/L	31.88%	0.01	0.01	0.01	0.02	8.20	0.05	0.28	0.01

Note that the substitution was applied on <LOD observations.

Table 2
Characteristics of MIREC participants who provided both a first trimester urine and blood sample (n = 1744).

Education	N	Percentage
High school or less	151	8.67%
College diploma	500	28.70%
Undergraduate university degree	636	36.51%
Graduate university degree	455	26.12%
Income ($)		
≤50,000	297	17.86%
50,001–100,000	686	41.25%
>100.000	680	40.89%
Country of birth		
Canada	1420	81.42%
Other	324	18.58%
Pre-pregnancy BMI		
≤18.5 (underweight)	57	3.32%
18.5–24 (normal)	1047	60.98%
25–29 (overweight)	373	21.72%
≥30 (obese)	240	13.98%
Parity		
0	775	44.44%
1	704	40.37%
2	200	11.47%
3+	65	3.73%
Smoking status		
Never	1063	61.02%
Former	472	27.10%
Quit during the pregnancy	105	6.03%
Current	102	5.86%

3.5. PC scores

Table 7 provides results of the Tukey post-hoc tests for the high-organochlorines component (PC1). As the low-phthalate (PC2) component did not indicate any significant differences at a 5% level of significance, no further analysis was conducted. Hypothesis test results for PC3 through PC11 are provided in Supplemental material, Tables S2–S10. Table 7 shows that the mean PC1 scores for some educational groups were significantly different from each other, with "undergraduate degree vs. college diploma" having the smallest mean difference, and "graduate degree *vs.* high school or less" having the largest mean difference. Pregnant women in the highest income group tended to have a significantly higher mean score than those in the middle and low income groups; however, no significant difference was noted between pregnant women in the low and middle income groups. The PC1 scores are also influenced by the birthplace of pregnant women, with higher scores for those born in Canada. The only two significant differences with respect to pre-pregnancy BMI were found between the overweight ($25 \le BMI < 30$) and normal groups ($18.5 \le BMI < 25$) and obese ($BMI \ge 30$) and normal groups. In addition, women who are pregnant for the first time (parity = 0) had a significantly higher mean score compared with those having one or more previous pregnancies. With respect to smoking status, significant differences were noted between current and never smokers, as well as between current and former smokers. Comparing the mean PC1 scores among the six clusters, the mean PC1 score of cluster 6 (born in Canada, high income,

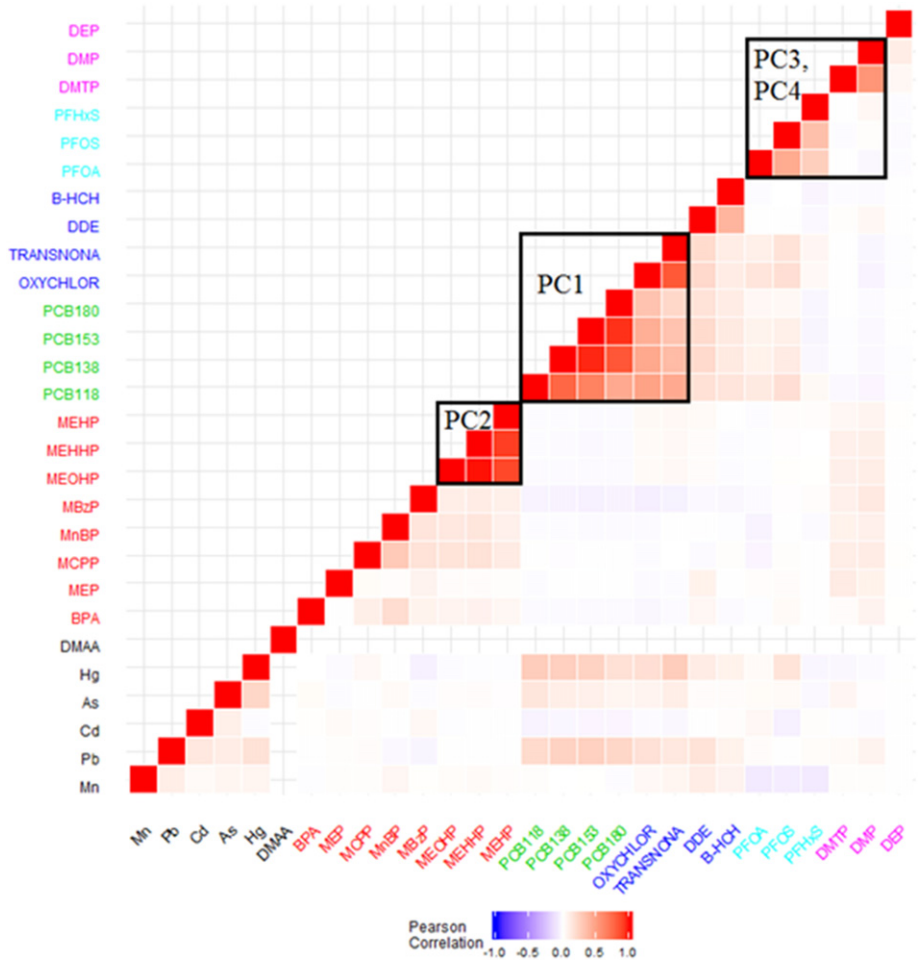

Fig. 1. Heat map of the Pearson correlation matrix of 28 chemicals.

Table 3
Relative frequency distributions (proportions) of demographic characteristic by cluster.

	Cluster					
Education	1	2	3	4	5	6
High school or less	0.01	0.04	0.07	0.20	0.28	0.49
College diploma	0.15	0.19	0.38	0.30	0.46	0.51
Undergraduate university degree	0.46	0.38	0.36	0.24	0.21	0.00
Graduate university degree	0.38	0.39	0.19	0.25	0.05	0.00
Income ($)						
≤50,000	0.00	0.16	0.00	0.45	1.00	0.00
50,001–100,000	0.00	0.38	0.97	0.36	0.00	0.11
>100.000	1.00	0.46	0.03	0.19	0.00	0.89
Birth place						
Canada	1.00	0.00	1.00	0.00	1.00	1.00
Non-Canada	0.00	1.00	0.00	1.00	0.00	0.00
Pre-pregnancy BMI						
≤18.5	0.02	0.03	0.03	0.07	0.04	0.06
18.5–24	0.67	0.92	0.55	0.05	0.53	0.31
25–29	0.21	0.00	0.23	0.74	0.21	0.34
≥30	0.09	0.05	0.18	0.13	0.23	0.29
Parity						
0	0.45	0.45	0.41	0.37	0.51	0.60
1	0.41	0.44	0.44	0.40	0.29	0.23
2	0.11	0.09	0.11	0.16	0.14	0.11
3+	0.03	0.02	0.04	0.07	0.06	0.06
Smoking status						
Never	0.69	0.71	0.58	0.75	0.41	0.17
Former	0.27	0.24	0.31	0.17	0.25	0.31
Quit during the pregnancy	0.03	0.03	0.08	0.04	0.10	0.17
Current	0.01	0.02	0.03	0.05	0.24	0.34
Number of participants	568	241	591	83	226	35

low education) was the highest, and was statistically higher than the mean scores of clusters 1 (born in Canada, high income, high education), 4 (born outside Canada, pre-pregnancy BMI at least 25) and 5 (born in Canada, low income). On the other hand, the mean PC1 score of cluster 4 was the lowest, and was statistically lower than the mean scores of clusters 1, 2 (born outside Canada, pre-pregnancy BMI <25), 3 (born in Canada, middle income) and 6.

Other findings are briefly summarized as follows: PC8 is dominated by all OCs, PFOA and two metals (Pb and Cd) and associated with the variables of education level, household income, country of birth, parity, maternal age, and the cluster. PC9 is only dominated by the metal Cd and associated with the education level, household income, country of birth, pre-pregnancy BMI, smoking status and cluster. PC11 is dominated by organophosphate pesticide DMP and plasticiser mono ethyl phthalate (MEP) and only associated by the characteristics of the pregnant women in terms of smoking status. The slope of the regression model of PC4, PC5, PC6 and PC8, individually, on maternal age is

Table 4
Five-number summary of maternal age for each cluster.

Cluster	Min	Q1	Median	Q3	Max
1	22	31	34	36	48
2	18	31	34	37	44
3	19	29	31	35	46
4	18	29	34	38	46
5	17	24	28	32	43
6	18	23.5	26	29	41

Table 5
The rotated eigenvectors of the eleven components after principal component analysis for 28 chemical substances in the first trimester from the MIREC Study.

Contaminant	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Mn	0.019	-0.0317	0.1655	-0.0981	0.2834	-0.0099	0.038	0.0261	0.1776	0.0275	-0.1683
Pb	0.1611	-0.02	0.1287	0.0162	0.1078	0.074	0.2013	0.3151	0.2795	0.1783	-0.1619
Cd	-0.0206	0.0051	0.042	0.0067	0.1151	-0.0436	0.185	0.4091	0.616	0.1826	0.1298
As	0.0811	-0.0389	0.12	0.0986	0.2037	-0.2015	0.4966	0.136	-0.1834	0.0342	0.0616
Hg	0.1862	-0.0141	0.0525	0.0873	0.2405	-0.2064	0.3168	0.0248	-0.3097	-0.0377	0.0192
DMAA	0.0474	-0.1609	0.2994	0.2132	0.1513	-0.106	0.199	0.0224	-0.2072	-0.2339	0.0972
BPA	-0.0184	-0.0789	0.1047	0.0876	-0.0296	-0.3113	-0.2279	0.0906	-0.0986	0.3178	-0.134
MEP	-0.0065	-0.0397	0.1272	0.1261	-0.0124	0.1602	-0.0846	0.1411	0.1232	-0.6309	0.3909
MCPP	-0.0083	-0.1669	0.1994	0.1337	-0.0558	-0.3934	-0.1993	0.011	0.0634	-0.0107	0.0347
MnBP	-0.0205	-0.1429	0.1914	0.0955	-0.0576	-0.4612	-0.2855	-0.0258	0.0445	0.1124	-0.0448
MEOHP	-0.017	-0.5421	-0.1422	-0.1169	-0.0184	0.064	0.0443	0.0217	-0.0149	0.0054	-0.0074
MBzP	-0.0533	-0.1143	0.1505	0.1384	-0.1222	-0.2003	-0.1919	0.0506	0.2343	-0.2295	0.2097
MEHHP	-0.0168	-0.5492	-0.1427	-0.1187	-0.0146	0.0503	0.0403	0.025	-0.0105	0.0057	0.0043
MEHP	-0.0141	-0.5151	-0.1736	-0.1348	-0.0073	0.0771	0.0416	0.0291	-0.0157	0.0063	0.0068
PFOA	0.0742	0.019	-0.3356	0.3924	-0.0532	0.0469	-0.0094	0.225	-0.0081	0.0328	0.0223
PFOS	0.0994	-0.0161	-0.3186	0.4627	-0.0208	-0.0475	-0.0427	0.135	-0.1801	-0.0241	-0.0012
PFHxS	0.0061	-0.002	-0.285	0.386	-0.077	0.0708	-0.0778	0.1796	0.0652	0.0794	-0.0732
PCB118	0.3833	-0.0026	-0.0329	0.0215	-0.0368	-0.0525	-0.0008	-0.0627	-0.0018	-0.0094	0.0164
PCB138	0.4431	0.0027	0.0483	-0.0984	-0.2462	-0.0108	-0.0016	0.0543	-0.0129	-0.0238	0.0023
PCB153	0.4361	0.0072	0.0682	-0.1246	-0.2879	0.0022	0.0027	0.0929	-0.022	-0.0183	-0.002
PCB180	0.3792	0.0117	0.0812	-0.1521	-0.3398	0.0238	-0.0065	0.1338	-0.0313	-0.0098	-0.0052
DMTP	-0.0148	-0.1255	0.3648	0.3156	-0.1384	0.3453	0.0608	-0.173	0.0431	0.0611	-0.2027
DMP	-0.0255	-0.1366	0.3751	0.3317	-0.1503	0.3328	0.026	-0.1272	0.003	0.1561	-0.1723
DEP	-0.0093	-0.0149	0.0876	0.0316	-0.0606	0.1264	0.0331	-0.1216	-0.0905	0.5171	0.7856
DDE	0.1546	-0.0393	0.1405	-0.0424	0.3595	0.2391	-0.3695	0.2522	-0.146	0.0241	0.0086
OXYCHLOR	0.3301	-0.0374	-0.1335	0.0992	0.2547	0.0147	-0.1001	-0.3819	0.2693	0.0211	0.0253
TRANSNONA	0.2964	-0.0511	-0.1053	0.1281	0.3489	-0.0332	-0.06	-0.43	0.218	-0.0046	0.0372
B-HCH	0.1054	-0.0002	0.102	-0.0838	0.3446	0.1952	-0.3888	0.2998	-0.2494	0.0813	0.0276
Eigenvalues	4.2091	3.0173	1.8364	1.7409	1.4825	1.3121	1.2271	1.202	1.0662	1.0158	0.9748
Variance explained (in %)	0.1503	0.1078	0.0656	0.0622	0.0529	0.0469	0.0438	0.0429	0.0381	0.0363	0.0348
Cumulative variance (in %)	0.1503	0.2581	0.3237	0.3858	0.4388	0.4857	0.5295	0.5724	0.6105	0.6468	0.6816

Note that the loadings highlighted in red are relatively large ineach column.

Table 6
p-Values for one way ANOVA tests where the mean component scores are equally likely from pregnant women groups: the MIREC Study.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Education	<0.001***	0.871	0.021**	0.768	0.817	0.919	0.494	<0.001***	<0.001***	0.534	0.123
Income ($)	<0.001***	0.387	0.001***	0.470	0.072*	0.129	0.414	<0.001***	<0.001***	0.425	0.293
Birth place	<0.001***	0.088*	<0.001***	0.000**	0.001***	0.021**	<0.001***	<0.001***	<0.001***	0.398	0.090
Pre-pregnancy BMI	<0.001***	0.499	0.086*	0.752	0.437	0.134	0.083*	0.106	0.028**	0.675	0.873
Parity	<0.001***	0.485	<0.001***	<0.001***	0.559	<0.001***	0.595	<0.001***	0.369	0.913	0.939
Smoking status	<0.001***	0.664	0.491	0.442	0.219	0.172	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Maternal age	<0.001***	0.539	0.123	<0.001***	<0.001***	0.001***	0.645	<0.001***	0.526	0.214	0.231
Clusters[a]	<0.001***	0.062*	<0.001***	<0.001***	<0.001***	0.075*	<0.001***	<0.001***	<0.001***	0.041**	0.363

[a] As obtained from the output of cluster analysis.
* Means the p-value is <10%.
** 5%.
*** 1%.

Table 7
Tukey's HSD tests for PC1.

	PC1			
	Difference	95% C.I.	p-Value	
Education				
College diploma - high school or less	−0.655	(−0.825, −0.485)	<0.001	***
Undergraduate university degree - high school or less	−0.784	(−1.043, −0.525)	<0.001	***
Graduate university degree - high school or less	−1.116	(−1.537, −0.694)	<0.001	***
Undergraduate university degree - college diploma	−0.129	(−0.391, 0.133)	0.586	
Graduate university degree - college diploma	−0.460	(−0.883, −0.037)	0.027	**
Graduate university degree - undergraduate university degree	−0.332	(−0.798, 0.134)	0.260	
Income ($)				
50,001 - 100,000 - ≤50,000	0.154	(−0.180, 0.488)	0.525	
>100.000 - ≤50,000	0.566	(0.231, 0.901)	<0.001	***
>100.000 - 50,001 - 100,000	0.412	(0.152, 0.672)	0.001	***
Birth place				
Canada - not-Canada	2.024	(1.796, 0.253)	<0.001	***
Pre-pregnancy BMI				
“18.5–24” - “≤18.5”	0.277	(−0.438, 0.992)	0.753	
“25–29” - “≤18.5”	−0.062	(−0.810, 0.686)	0.997	
“≥30” - “≤18.5”	−0.417	(−1.192, 0.358)	0.510	
“25–29” - “18.5–24”	−0.339	(−0.656, −0.021)	0.031	**
“≥30” - “18.5–24”	−0.693	(−1.070, −0.316)	<0.001	***
“≥30” - “25–29”	−0.355	(−0.790, 0.081)	0.156	
Parity				
“1” - “0”	−0.438	(−0.710, −0.166)	<0.001	***
“2” - “0”	−0.671	(−1.086, −0.256)	<0.001	***
“3 +” - “0”	−1.186	(−1.860, −0.512)	<0.001	***
“2” - “1”	−0.233	(−0.653, 0.186)	0.480	
“3 +” - “1”	−0.748	(−1.425, −0.071)	0.023	**
“3 +” - “2”	−0.515	(−1.261, 0.231)	0.286	
Smoking status				
Former - never	0.091	(−0.199, 0.381)	0.852	
Quit during the pregnancy - never	−0.407	(−0.944, 0.129)	0.207	
Current - never	−0.922	(−1.469, −0.376)	<0.001	***
Quit during the pregnancy - former	−0.498	(−1.064, 0.068)	0.107	
Current - former	−1.013	(−1.588, −0.438)	<0.001	***
Current - quit during the pregnancy	−0.515	(−1.246, 0.216)	0.268	
Clusters[a]				
“2” - “1”	1.844	(1.182, 0.506)	<0.001	***
“3” - “1”	1.177	(0.524, 1.831)	<0.001	***
“4” - “1”	−0.802	(−1.174, −0.430)	<0.001	***
“5” - “1”	−0.558	(−0.891, −0.225)	<0.001	***
“6” - “1”	1.872	(1.430, 0.315)	<0.001	***
“3” - “2”	−0.667	(−1.555, 0.222)	0.267	
“4” - “2”	−2.646	(−3.353, −1.939)	<0.001	***
“5” - “2”	−2.402	(−3.089, −1.715)	<0.001	***
“6” - “2”	0.028	(−0.718, 0.775)	1.000	
“4” - “3”	−1.979	(−2.678, −1.280)	<0.001	***
“5” - “3”	−1.735	(−2.415, −1.056)	<0.001	***
“6” - “3”	0.695	(−0.044, 1.434)	0.079	*
“5” - “4”	0.244	(−0.172, 0.659)	0.549	
“6” - “4”	2.674	(2.167, 3.182)	<0.001	***
“6” - “5”	2.431	(1.951, 1.910)	<0.001	***

[a] Cluster 1 included women born in Canada with a high income level and high education level; Cluster 2 included women born outside of Canada and with a pre-pregnancy BMI lower than 25; Cluster 3 included women born in Canada with a middle income level; Cluster 4 included women who were born outside of Canada and with a pre-pregnancy BMI at least 25; Cluster 5 included women born in Canada with a low income level; and, Cluster 6 included women born in Canada with a high income level and low education level.

* Means the p-value is <10%.
** 5%.
*** 1%.

significant. In terms of cluster analysis results, women in clusters 1 and 5 have a significant high level of PC8 (dominated by all OCs, PFOA, Pb and Cd) than the rest. Also, women in cluster 4 have a significantly higher level of cadmium among all six clusters.

3.5.1. PC3 and PC4 scores

PC3 and PC4 scores were dominated by the same chemical mixtures (dimethylarsinic acid (DMAA), dimethylthiophosphate (DMTP), dimethylphosphate (DMP), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS), as shown in Table 5) and all dominating values of the corresponding eigenvectors for the chemicals were positive, with the exception of PFOA, PFOS, and PFHxS for PC3. Intuitively, PC4 should be a better component to identify the association between the scores and the characteristics of the participants since almost all of its loadings are positive, suggesting that higher scores indicate higher exposure. The fact that PC3 has some negative and some positive values is more difficult to interpret; however, the p-values for many of the associations of PC3 with socio-demographic characteristics are significant. In an effort to explain these results, scatterplots of PC3 and PC4 scores by socio-demographic variables were created (Fig. 2). These show a moderate negative linear correlation between the PC3 and PC4 scores. Further investigations (Supplemental material, Figs. S1–S3) demonstrated that, given the characteristics of the participants, the participants who had higher concentrations of PFOA, PFOS, and PFHxS had relatively lower concentrations for DMAA, DMTP and DMP. For example, in Fig. S1 those with

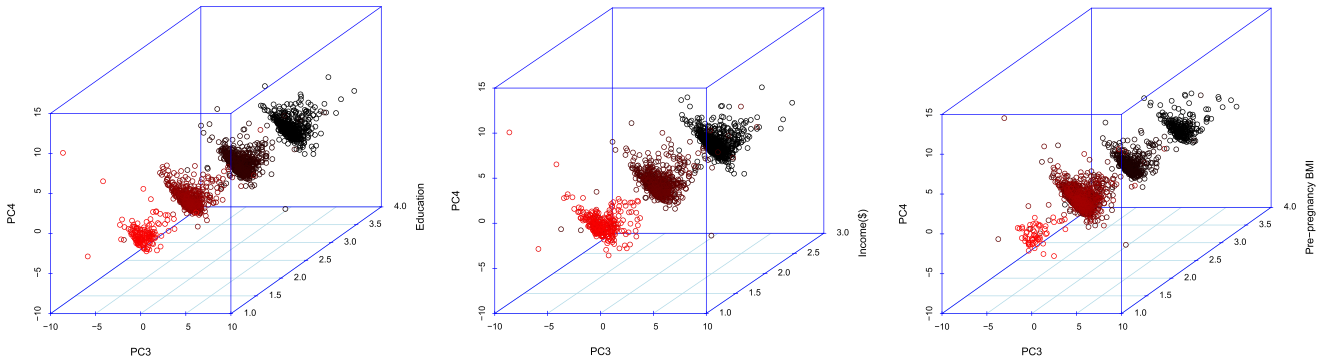

Fig. 2. Scatterplots of PC3 and PC4 scores by socio-demographic variables.

high school education or less have the highest mean chemical levels in DMAA, DMTP and DMP but the lowest mean chemical levels in PFOA, PFOS, and PFHxS.

4. Discussion

A longstanding and complex issue is how to evaluate and consequently limit exposure to chemical mixtures during pregnancy. Humans are frequently exposed to multiple chemicals and stressors simultaneously; however, previous analyses of MIREC data (Arbuckle et al., 2014; Arbuckle et al., 2015; Ashley-Martin et al., 2015; Colapinto et al., 2015; Shapiro et al., 2015; Thomas et al., 2015; Vélez et al., 2015) have investigated either exposure to or potential adverse health effects of environmental chemicals on pregnancy and infant health but with a focus on individual chemicals. Recognizing that single chemical models cannot reflect the real world of complex chemical mixtures, the present statistical analysis identified chemical mixtures and investigated the impact of socio-demographics on the type of mixtures to which pregnant women are exposed to help identify patterns of exposure to multiple chemicals. The results of cluster analysis described the selected seven socio-demographic variables simultaneously and statistical differences were noted.

Kim et al. (2015) applied PCA to analyze a series of heavy metals and POPs. Scatterplots of the loadings of the components were used to examine the prenatal exposure pattern; however, this method is questionable since the loadings of the components should be used to convert the data into scores for further analysis. Agay-Shay et al. (2015) collected data from 27 endocrine-disrupting chemicals and used PCA to examine the association between the prenatal exposures and characteristics of children at 7 years old. Four principal components were generated that accounted for 43.4% of the total variance in the data. For each of the components, the participants were divided into three groups based on the factor scores and the association between the characteristic and exposure were examined within tertiles. Robinson et al. (2015) evaluated 81 chemicals (also categorized into 13 exposure families) in blood/urine samples obtained throughout pregnancy for 728 women in the INMA birth cohort during 2004 to 2006 and applied PCA to each exposure family and across all 81 exposures. Only the number of components required to explain certain percentages of cumulative variance by each exposure family and across all 81 exposures individually were reported in their study, and a detailed analysis by demographic variables was not included. Veyhe et al. (2015) analyzed 22 chemicals (eight PCBs, four OCs, five essential and five toxic elements) in serum or whole blood of pregnant women recruited as part of the MISA Study in Northern Norway along with the characteristics of the participants. The first six PCA components which accounted for 74% of the source variation were kept for further analysis. Multiple linear regressions were adopted for modeling the relationship between the components and participants' characteristics; however, the values of the coefficients of the determinations were not high (ranged from 0.04 to 0.426). The advantages of using linear regressions are to build a model for predictive purpose, while the disadvantage is the inability of the method to evaluate detailed pairwise comparisons. Our results were most similar to those reported by Veyhe et al. (2015) as chemical concentrations were found to have some associations with maternal age, parity and pre-pregnancy BMI. Other studies using principal component analysis have shown that POPs dominate one component which is consistent with our results (Kim et al., 2015; Agay-Shay et al., 2015; Robinson et al., 2015; Veyhe et al., 2015).

By combining some results from both Table 5 and the correlation matrix (as shown in Fig. 1), the PCA results also captured the linear correlation structure among the chemicals. Six chemicals (PCB118, PCB138, PCB153, PCB180, OXYCHLOR and TRANSNONA) that dominated PC1 are relatively highly and linearly correlated and the largest subgroup among the 28 chemicals. PCB118, PCB138, PCB153, PCB180, OXYCHLOR and TRANSNONA are persistent organic pollutants, where the major source is meat and dairy. The highest concentrations are found in animals at the top of the food chain, including humans (Health Canada, 2005, 2010). Therefore, we were not surprised to observe that these chemicals were highly correlated and dominate one component.

Three phthalates (MEOHP, MEHHP AND MEHP) that dominated the second component are also highly and linearly correlated. MEOHP, MEHHP and MEHP are the metabolites of di-2-ethylhexyl phthalate (DEHP) (Koch et al., 2003); hence, one would expect them to be clustered together. DEHP is widely used in food packaging, cosmetics and personal care products including fragrances, soft PVC products, building and furniture materials, and medical devices (Zarean et al., 2016). DEHP has been one of the most important plasticizers used in Canada (Environment Canada, Health Canada, 1994), so it is not surprising that human exposure to DEHP is nearly ubiquitous (Environment & Human Health, Inc., 2008). In our study MEOHP, MEHHP and MEHP were found in >98% of the urine samples. Instead of examining if pregnant women are highly exposed to a certain chemical, PCA allowed us to examine whether pregnant women were highly exposed to a certain group of chemicals. A drawback of the principal component analysis is the difficulty of interpretation when the components have both large positive or small negative eigenvectors, as it is unable to decide which chemicals define the particular component. For the same reason it is also difficult to name the components. For cluster analysis, results may differ due to different choices of the dissimilarity matrix and linking algorithms; however, sensitivity analysis using various approaches may be used to help interpret results.

There are a number of limitations in our analysis. For chemical levels below the limit of detection, we substituted a constant (LOD/2) in order to use standard statistical methods. This substitution may lead to issues of bias and underestimated variance in hypothesis testing (Helsel, 2006; Nie et al., 2010; Nysen et al., 2012). Imputation methods, such as regression on order statistics (Helsel, 2012) or multiple imputation by chained equations (White et al. 2011; Royston and White, 2011), are available. However, regression on order statistics is suitable for a small data set

for which all nondetects are ordered and multiple imputations by chained equations require highly correlated variables. Despite these methods, further development of statistical methods to account for non-detects in multivariate analysis is a worthy endeavour. Further, only one urine sample is used to measure non-persistent chemicals which may result in measurement error.

In conclusion, our results show the association between certain socio-demographic characteristics of the population of pregnant women and the presence of residual mixtures of common chemicals in their blood and urine. The identification of patterns of chemicals and associated patterns of pregnant women with high exposures using advanced statistical approaches is an important first step of analysis. Future research would benefit from examining the effect of chemical mixtures identified in this type of analysis on the potential for adverse health effects in pregnant women or their children, in order to better inform risk assessments. Last but not least, other statistical approaches, for example a nonlinear model or a linear model including interactions between covariates, may also be considered in future analysis of chemical mixtures.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.12.015.

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