Using Latent Profile Analysis to Identify Associations Between Gestational Chemical Mixtures and Child Neurodevelopment

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Background: Unsupervised machine learning techniques have become increasingly popular for studying associations between gestational exposure mixtures and human health. Latent profile analysis is one method that has not been fully explored.

Methods: We estimated associations between gestational chemical mixtures and child neurodevelopment using latent profile analysis. Using data from the Maternal-Infant Research on Environmental Chemicals (MIREC) research platform, a longitudinal cohort of pregnant Canadian women and their children, we generated latent profiles from 27 gestational exposure biomarkers. We then examined the associations between these profiles and child Verbal IQ, Performance IQ, and Full-Scale IQ, measured with the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III). We validated our findings using k-means clustering.

Results: Latent profile analysis detected five latent profiles of exposure: a reference profile containing 61% of the study participants, a high monoethyl phthalate (MEP) profile with moderately low persistent organic pollutants (POPs) containing 26%, a high POP profile containing 6%, a low POP profile containing 4%, and a smoking chemicals profile containing 3%. We observed negative associations between both the smoking chemicals and high MEP profiles and all IQ scores and between the high POP profile and Full-Scale and Verbal IQ scores. We also found a positive association between the low POP profile and Full-Scale and Performance IQ scores. All associations had wide 95% confidence intervals.

Conclusions: Latent profile analysis is a promising technique for identifying patterns of chemical exposure and is worthy of further study for its use in examining complicated exposure mixtures.

Keywords: Child health; Latent profile analysis; Maternal exposures; Unsupervised machine learning; Wechsler scales

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Neurotoxicant exposure during the gestational period can severely impact cognitive development.[1–4] Although the effects of many gestational neurotoxicants have been well documented, most studies focus on one chemical at a time, or restrict their models to toxicants in the same chemical group.[1,3,5–11] These studies are not reflective of real-world exposures, where people encounter a complex mixture of chemicals from different classes every day.[12] By studying chemicals individually or in small groups, researchers may underestimate the collective impact of exposure to multiple groups of chemicals and fail to identify interactions between toxicant effects.[13] If there are high levels of correlation between exposure variables, studying these chemicals together in simple models can result in large standard errors if one does not account for collinearity.[4,14,15] Multiple statistical methods exist for studying chemical mixtures, including unsupervised techniques such as k-means clustering, hierarchical clustering, and principal component analysis (PCA); however, the best method is unclear.[2,5,9,15,16]

Latent profile analysis is a model-based technique commonly used in psychology and behavioral sciences.[17–21] It is designed to detect patterns in continuous independent variables, which are used to create homogenous subgroups called profiles. The method has been introduced to, but not fully explored in, the field of environmental epidemiology. In 2021, Khorrami et al.[22] showed that it had promise as a method for

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All code for this project can be accessed at https://github.com/amyonkman/LPA-Paper-Code.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).

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studying chemical mixtures, and studies have also used a similar technique called latent class analysis for this purpose.[23–24] Latent profile analysis has been shown to have advantages compared with other methods such as k-means clustering, a popular clustering technique that has often been used to study chemical mixtures in environmental epidemiology.[2,25–27] Unlike k-means clustering, latent profile analysis generates profiles that are probabilistic, generating the posterior probabilities that each observation will fall into each profile.[28] Posterior probabilities range from 0 to 1, with 1 denoting a perfect match between observation and profile. This provides more nuanced results than nonprobabilistic clustering methods such as k-means clustering because it allows researchers to assess classification accuracy.[17–19] Also, latent profile analysis does not have a bias toward creating groups of the same size, which k-means clustering does.[29,30] Finally, the method does not require the researcher to predetermine the number of profiles and has more rigorous methods of choosing a model than k-means clustering.[19,20,28]

The aim of this study was to use latent profile analysis to create profiles of gestational chemical exposures in pregnant Canadian women based on 27 biomarkers and to examine associations between profile membership and cognitive scores. We validated our findings in a sensitivity analysis using k-means clustering, a more established unsupervised technique.

METHODS

Study Participants

We used data from the Maternal-Infant Research on Environmental Chemicals (MIREC) study, an ongoing pregnancy cohort that began in 2008.[31–33] The primary objective of the MIREC study is to examine associations between gestational chemical exposures, measured using biomarkers in maternal blood and urine, and various health outcomes. Approximately 2000 participants from 10 Canadian cities were included in the original study (eFigure 1; http://links.lww.com/EDE/B972). Details about eligibility and exclusion criteria are outlined by Arbuckle et al.[32] At age 36–48 months, a subset of 610 participating children in six cities were included in a follow-up study in which researchers assessed neurodevelopment.[3,34–36] We restricted our study to 517 mother–child pairs from this group based on the availability of biomarkers and intelligence quotient (IQ) scores.[33,35,36]

The MIREC study received ethics approval from Health Canada and the Institutional Review Board of CHU Sainte-Justine Research Centre, as well as all recruitment centers. We also received approval from the Simon Fraser University office of research ethics (REB Number: 20160678). All participants gave written informed consent.

Biomarkers of Gestational Toxicant Exposure

We studied 27 potential neurotoxicants measured in the first trimester: five metals, four organochlorine pesticides (OCPs), five organophosphate pesticide metabolites (OPPs), four phthalate metabolites plus the molar sum of Bis(2-ethylhexyl) phthalate (DEHP) (comprised of mono-[2-ethyl-5-hydroxyhexyl] phthalate, mono-[2-ethylhexyl] phthalate, and mono-[2-ethyl-5-oxohexyl] phthalate), six polychlorinated biphenyls (PCBs), one polybrominated diphenyl ether (PBDE), and cotinine (Table 1). We measured the metals, OCPs, PCBs, PBDE, and cotinine in maternal blood, and the OPPs and phthalates in maternal urine.[32] Samples were collected between 6 and 13 weeks of gestation, then quantified using gas chromatography/mass spectrometry at the Institut national de santé publique du Québec. We standardized the OCPs, PCBs, and PBDE using the total plasma-lipid concentrations to account for lipid dilution, and standardized the OPPs and phthalates using specific gravity to account for urine dilution, as described by Hu et al.[15,37,38]

Each biomarker had a limit of detection (LOD) below which it could not be measured; for chemical measures that fell <LOD, we employed a single imputation "fill-in" method described by Lubin et al.[39] We first $\log_2$-transformed our chemicals to reduce skewness, then temporarily replaced the values <LOD with LOD/√2. We used these values to determine the mean and standard deviation of a truncated lognormal distribution. We replaced the values <LOD with values randomly sampled from this distribution. We excluded chemicals with greater than 60% of observations <LOD.

Child Cognitive Abilities

We assessed cognitive abilities using the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III) when the children were on average 3.4 years old (range: 2.8–4.2).[1,3,35,36] The test was administered by trained examiners, typically in the children's homes. The WPPSI-III test generates a Verbal IQ (VIQ) score, which measures acquired knowledge and verbal abilities; a Performance IQ (PIQ) score, which measures nonverbal reasoning, spatial processing, attention, and visual-motor coordination; and a Full-Scale IQ (FSIQ) score, which measures overall cognitive abilities.[40–42] All three IQ scores are scaled to a Canadian reference population with a mean of 100 and a standard deviation of 15. Higher scores indicate greater cognitive abilities.

Statistical Analysis

Descriptive Statistics

After selecting our cohort from the MIREC study population, we assessed the central tendency and distribution of the mothers' gestational biomarker concentrations. We compared these values to the exposure levels measured in Cycle 1 of the Canadian Health Measures Survey (CHMS; 2007–2009) to ensure that our results reflected those of the Canadian population.[43] We also assessed the distribution of the children's WPPSI-III scores in the total group and by demographic, and then examined associations between the individual $\log_2$-transformed chemical concentrations and WPPSI-III scores.

Latent Profile Analysis

We used latent profile analysis to create chemical mixture profiles based on 27 chemical biomarkers. We first conducted Pearson pairwise correlation analysis on the $\log_2$-transformed biomarkers, then performed latent profile analysis with the R packages tidyLPA (tidyLPA: An R Package to Easily Carry Out Latent Profile Analysis [LPA] Using Open-Source or Commercial Software, Knoxville, Tennessee, USA) and mclust (Gaussian Mixture Modelling for Model-Based Clustering, Classification, and Density Estimation, Perugia, Italy; for information on how to access project code, see Supplementary Materials; http://links.lww.com/EDE/B972).[44] In total, we generated 36 models with up to 12 profiles and one of the following three sets of assumptions: equal variance and no covariance, varying variance and no covariance, and equal variance and covariance (eTable 3; http://links.lww.com/EDE/B972). We used three quality measures to assess these models. First, we calculated the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC). These measures increase with model complexity and decrease with model validity; the goal is to achieve the smallest BIC and AIC possible.[45] For each model, we also calculated entropy, assessing the confidence with which individuals were classified in each group (our cutoff for an acceptable model was 0.8).[46] Finally, note that we considered model interpretability (as recommended by Spurk et al.[46]), preferring models with more visibly distinct exposure profiles.

After determining the number of profiles, we generated the mothers' posterior probabilities of profile membership. We then examined the demographic characteristics of participants in each profile. Additionally, we calculated the profiles' mean chemical concentrations using the formula:

$$W = \frac{\sum P_i X_i}{\sum P_i}$$

where X_i is the $\log_2$-transformed chemical concentration in mother i and P_i is the posterior probability of profile membership in mother i. We converted the mean biomarker concentrations to z-scores to determine the patterns detected by latent profile analysis.

Covariates

Trained MIREC staff obtained data on potential confounders by administering standardized interviews and questionnaires to the pregnant mothers.[3,34,47] To choose covariates for this study, we constructed a directed acyclic graph (DAG) using information from established literature (eFigure 2; http://links.lww.com/EDE/B972).[48–51] We adjusted for maternal age, race, education, marital status, household income, parity, prenatal alcohol exposure, Home Observation Measurement of the Environment (HOME scores; split into quartiles), and city of recruitment. We did not adjust for self-reported prenatal smoking because cotinine was a factor in profile generation. Gestational age and birth weight were excluded from the model because they act as mediators, not confounders.[52] Although we did not consider child sex a confounder, we stratified results by sex because studies have estimated differing effects of gestational exposures on neurodevelopment in boys and girls.[1,3,8] We also ran regression models that included all main effects of probability of profile membership as well as all two-way sex by probability of profile membership interaction terms, to systematically examine sex-specific associations.

Measures for several of our covariates were missing in a small number of participants. Prenatal alcohol was missing for 4.1% of mothers, household income for 3.5%, HOME scores for 3.3%, and maternal education for 0.4%. To mitigate this, we imputed multiple sets of values for each missing number using the R package *mice* (Multivariate Imputation by Chained Equations, Utrecht, Netherlands), then chose the single set of values that least impacted the frequency of each variable (mean for continuous variables or percentage for categorical variables).

Regression Analysis of the Latent Profiles

We used covariate-adjusted multiple linear regression analysis to measure the associations between profile membership and WPPSI-III scores, running separate regression models for VIQ, PIQ, and FSIQ. Each model included the posterior probabilities of profile membership for every profile except the reference, as shown in the following equation:

$$Y = \beta_0 + \beta_2 Z_2 + \ldots + \beta_k Z_k + \beta_{c1} C_1 + \ldots + \beta_{cp} C_p \quad (1)$$

where Y is the WPPSI-III score, $Z_2 \ldots Z_k$ are the posterior probabilities that a mother will fall into each of the k profiles, Z_1 is the reference profile that is excluded from the model, and $C_1 \ldots C_p$ are p confounders. For example, the quantity β_3 would be the change in mean IQ score as the posterior probability of membership in profile 3 increases from 0 to 1, implying a 100% probability of inclusion in profile 3 compared with profile 1, adjusted for p confounders.

Sensitivity Analysis Comparing Latent Profile Analysis With K-means Clustering

We conducted sensitivity analysis comparing latent profile analysis with k-means clustering. Unlike latent profile analysis, k-means clustering requires that the researcher predetermine the number of clusters.[53,54] We first ran several models and used the elbow method, assessing the total within-cluster sum of squares, to confirm our choice. We generated a heat map of mean biomarker concentration z-scores and compared them with the latent profile analysis results.

We used covariate-adjusted multiple linear regression analysis to measure the associations between cluster membership and WPPSI-III scores, once again running separate models for VIQ, PIQ, and FSIQ. We created indicator variables for each of the clusters and then repeated the analysis shown in Equation 1, replacing the posterior probabilities with these indicator variables.

TABLE 1. Distribution of Gestational Chemical Biomarkers in Participating Mothers in the MIREC Study During Their First Trimester of Pregnancy, Compared to the Geometric Mean Concentrations Found in the Canadian Mothers in Cycle 1 (2007–2009) of the CHMS (n = 517)[36]

Toxicant	% >LOD	GM (GSD)	Percentiles					CHMS GM
			25th	50th	75th	95th	Max	
Metals—whole blood (ug/L)								
Arsenic	96.5	0.8 (2.0)	0.6	0.8	1.2	2.3	34.5	0.9
Cadmium	97.5	0.2 (2.1)	0.1	0.2	0.3	0.7	5.1	0.4
Lead	100.0	6.4 (1.6)	4.6	6.2	8.5	14.1	41.4	8.9
Manganese	100.0	8.7 (1.4)	7.1	8.8	10.4	13.7	26.9	9.8
Mercury	91.5	0.6 (2.7)	0.4	0.7	1.3	2.8	7.8	0.7
OCPs—plasma (ng/L)								
β-HCH	64.0	2.3 (2.7)	< LOD	2.1	3.4	9.0	500.0	4.8
DDE	99.2	55.23 (2.2)	35.7	49.1	77.1	214.6	2,656.3	102.2
Oxychlor	92.8	2.0 (1.8)	1.5	2.2	3.0	4.5	8.4	2.3
trans-nonachlor	85.7	2.9 (1.8)	2.0	3.0	4.3	7.4	18.3	3.1
OPPs—urine (ug/L)								
DEP	73.1	2.6 (2.3)	< LOD	2.5	4.2	9.8	2,104.8	2.0
DETP	49.1	0.7 (2.5)	< LOD	< LOD	1.2	3.1	15.6	NA
DMDTP	53.0	0.5 (3.6)	< LOD	0.5	1.1	4.8	22.5	NA
DMP	77.4	3.2 (2.7)	1.8	3.3	6.2	14.7	71.5	2.6
DMTP	80.7	3.6 (4.0)	1.4	3.9	8.9	30.6	96.2	1.8
Phthalates—urine (ug/L)								
MnBP	99.6	12.6 (2.3)	7.8	12.1	19.2	47.1	525.9	18.0
MBzP	99.4	5.4 (2.5)	3.1	4.8	9.1	25.3	182.0	9.3
MCPP	80.3	0.9 (3.3)	0.5	0.9	1.7	7.2	72.0	1.1
MEP	100.0	33.2 (4.0)	12.4	26.0	71.5	416.0	20,800.0	50.0
DEHP sum (nmol/L)	NA	18.6 (2.1)	12.5	18.1	26.4	63.8	550.6	NA
PCBs—plasma (ng/L)								
PCB118	77.0	2.4 (1.9)	1.7	2.5	3.4	6.6	30.2	3.1
PCB138	94.2	4.5 (2.0)	2.9	4.5	6.6	15.1	46.8	5.5
PCB153	99.8	8.0 (2.0)	5.0	7.6	11.8	27.9	80.9	8.2
PCB170	58.8	2.0 (2.2)	< LOD	2.0	3.1	8.1	40.3	NA
PCB180	96.7	5.5 (2.2)	3.2	5.3	8.4	21.2	114.9	5.8
PCB187	47.2	1.7 (2.0)	< LOD	< LOD	2.5	5.7	26.9	NA
PBDEs—plasma (ng/L)								
BDE47	65.0	7.3 (2.7)	< LOD	6.8	11.7	38.1	727.3	10.8
Tobacco metabolites—plasma (ng/L)								
Cotinine	54.4	7.7 (11.8)	<LOD	6.5	20.0	270.0	180,000.0	NA

OCPs, PCBs, and PBDEs are standardized using total lipids, and OPPs and phthalates are standardized using specific gravity.

DDE indicates dichlorodiphenyldichloroethylene; DEP, diethylphosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; GM, geometric mean; GSD, geometric standard deviation; MBzP, monobenzyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MnBP, monobutyl phthalate; NA, not available; β-HCH, β-benzene hexachloride.

RESULTS

Study Participants and Descriptive Statistics

Our study population consisted of 517 mother–child pairs with complete information on all 27 biomarkers and complete WPPSI-III scores (Table 1; Table 2; eFigure 1; http://links.lww.com/EDE/B972). When we compared our sample to the average Canadian mother, as measured by Statistics Canada in 2011, we found that our participants tended to be older (41% ≥35), more likely to be White (86%), with higher socioeconomic status (68% with undergraduate degree or higher; 41% with a household income of ≥$100,000/year) and lower self-reported prenatal smoking (9%) and alcohol consumption rates (17%; Table 2).[55] Our study population included participants in Vancouver, Toronto, Hamilton, Kingston, Montreal, and Halifax. The largest proportion came from Montreal or Kingston (24% each) and the smallest from Vancouver (9%). These patterns were similar to those in the original MIREC cohort.[25] Mean IQ scores were higher in females and children with White, educated, low-parity, or nonsmoking mothers, or those who had higher HOME scores or tested in Vancouver or Hamilton (eTable 1; http://links.lww.com/EDE/B972). Self-reported

TABLE 2. Demographic Characteristics for Total Study Population and for Participants in Each Profile

Demographic Category	Total (%) N = 517	Reference (%) N = 314	High MEP (%) N = 136	High POP (%) N = 30	Low POP (%) N = 19	Smoking Chemicals (%) N = 18
Child sex						
Male	49	49	51	53	26	50
Female	51	51	49	47	74	50
Maternal age						
19–30	21	12	32	13	58	44
30–35	39	41	40	30	21	22
35+	41	46	28	57	21	33
Maternal race						
White	86	88	83	80	79	89
Other	14	12	17	20	21	11
Maternal education						
High school	5	1	10	7	26	22
College	27	26	30	23	26	39
Undergraduate	38	39	37	40	47	39
Graduate	29	34	24	30	0	0
Marital status						
Married	72	73	76	70	53	56
Unmarried	28	27	24	30	47	44
Household income (Canadian Dollars)						
< 40,000	9	7	9	10	26	33
40,000–80,000	29	28	30	33	32	22
80,000–100,000	21	21	23	13	26	11
> 100,000	41	44	38	43	16	33
Parity						
Zero	44	43	45	37	42	50
One	41	44	36	50	26	33
Two	12	11	13	13	16	17
Three or more	3	2	7	0	16	0
Prenatal smoking						
No	91	96	90	93	100	11
Yes	9	4	10	7	0	89
Prenatal alcohol						
No	83	82	87	90	89	61
Yes	17	18	13	10	11	39
HOME score quartile						
1st	26	22	30	37	37	44
2nd	28	28	27	30	26	17
3rd	22	24	21	17	16	17
4th	24	26	22	17	21	22
Test site						
Vancouver	9	11	4	20	5	6
Toronto	10	12	7	7	0	6
Hamilton	13	12	15	13	21	6
Kingston	24	21	29	13	26	39
Montreal	24	24	22	43	32	22
Halifax	20	20	22	3	16	22

prenatal alcohol consumption and frequency of prenatal drinking were positively associated with child IQ. However, given the low levels of alcohol consumption in mothers who reported prenatal drinking (median = one drink per month), this may have been driven by socioeconomic factors. Finally, the geometric mean biomarker concentrations in our sample were similar to those in cycle 1 of the CHMS (2007–2009; Table 1).[43]

Latent Profile Analysis

We created a heat map showing the correlation between pairs of $\log_2$-transformed biomarkers (Figure 1).

We found high correlation between chemicals in the same group, particularly in the OCPs, OPPs, PCBs, and phthalates. There was also correlation between certain chemicals in different groups; the PCBs, OCPs, and most of the metals were correlated with one another, as were cotinine and cadmium.

For the latent profile analysis, we generated 36 models in total (eTable 3; http://links.lww.com/EDE/B972). The AIC got steadily lower with increasingly complex models, with the lowest value for a model with 12 profiles, and was therefore not used for model choice. The BIC was lowest in models with equal variance and covariance. Of these, the two models with the lowest BIC had five profiles (BIC = 39,625) and eight profiles (BIC = 39,566). Both of these profiles had entropy values above 0.8 (0.885 and 0.899, respectively). The five-profile model was chosen for the sake of interpretation, which gets more difficult as the number of profiles increases. When we examined this model, we found that it contained a reference profile, which had z-scores of biomarker concentrations close to zero; a high monoethyl phthalate (MEP) and moderately low persistent organic pollutant (POP) profile (for brevity's sake, we will call this the high MEP profile), which had moderately low concentrations of all chemicals except for phthalates and slightly higher levels of MEP; a high POP profile, which had very high concentrations of PCBs; a low POP profile, which had very low concentrations of PCBs and OCPs; and a smoking chemicals profile, which had high concentrations of cotinine and cadmium biomarkers. We examined the number of people who most closely matched each group and found that it varied between profiles, with the reference and high MEP profiles being larger (n = 314 and 136, respectively) and high POP, low POP, and smoking chemicals profiles being smaller (n = 30, 19, and 18, respectively). There was high variance of PCB and cotinine concentrations between the five profiles, and lower variance in the other chemical groups (Figure 2). Mothers in the three smaller profiles had high average posterior probabilities (mean >0.98), whereas mothers in the reference and high MEP profiles had lower average posterior probabilities (mean = 0.92 and 0.89, respectively).

The high POP profile had older, non-White mothers (eTable 5; http://links.lww.com/EDE/B972). The low POP profile had younger, non-White, unmarried mothers with lower socioeconomic status, many of whom tested from Montreal and Hamilton, and who gave birth to a higher proportion of girls. The smoking chemicals profile had younger, non-White, unmarried mothers with lower household income and education and higher levels of active smoking, although not all mothers in this profile reported prenatal smoking.

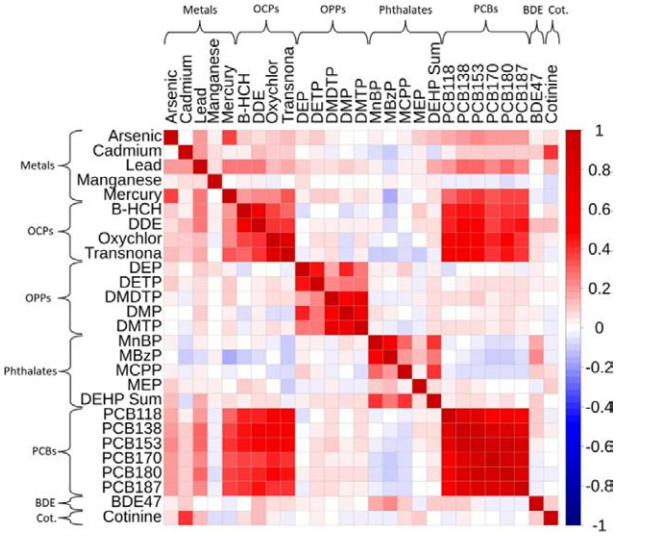

FIGURE 1. Pearson correlation heat map of 27 log2-transformed chemicals measured in participating mothers in the MIREC study during their first trimester of pregnancy (n = 517). BDE indicates brominated diphenyl ether; DDE, dichlorodiphenyldichloroethylene; DEP, diethylphosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; MBzP, monobenzyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MnBP, monobutyl phthalate; β-HCH, β-benzene hexachloride.

Regression Analysis of the Latent Profiles

The high MEP and smoking chemicals profiles were negatively associated with all three scores in all children when compared to the reference profile (FSIQ: –1.7 [–4.8, 1.4] and –2.6 [–8.8, 3.7], respectively), although the smoking chemicals profile showed a much higher association in female children and more neutral associations in male children (Figure 3). The high POP profile showed negative associations with VIQ (–1.8 [–6.3, 2.8]) but neutral associations with PIQ (0.0 [–5.4, 5.4]). In the low POP profile, we found a positive association with PIQ scores (4.5 [–2.5, 11.5]) but a negative association with VIQ scores (–2.0 [–7.9, 3.9]). For all regression coefficients, the 95% confidence intervals covered the null value. However, the effect estimates were often much higher than those of the individual chemicals we studied (eTable 2; http://links.lww.com/EDE/B972 and eTable 4; http://links.lww.com/EDE/B972). We found no statistically significant interactions between latent profiles and child sex.

Sensitivity Analysis Comparing Latent Profile Analysis and K-means Clustering

Using k-means clustering, we found that 5–8 clusters were most appropriate. We chose a model with five clusters to compare with the latent profile analysis results. Four of the clusters were similar to the profiles generated by latent profile analysis; Figure 4 shows a cluster with low- to mid-level biomarker concentrations, one with high biomarker concentrations, one with low biomarker concentrations except for the phthalates (in this case, phthalates were all relatively

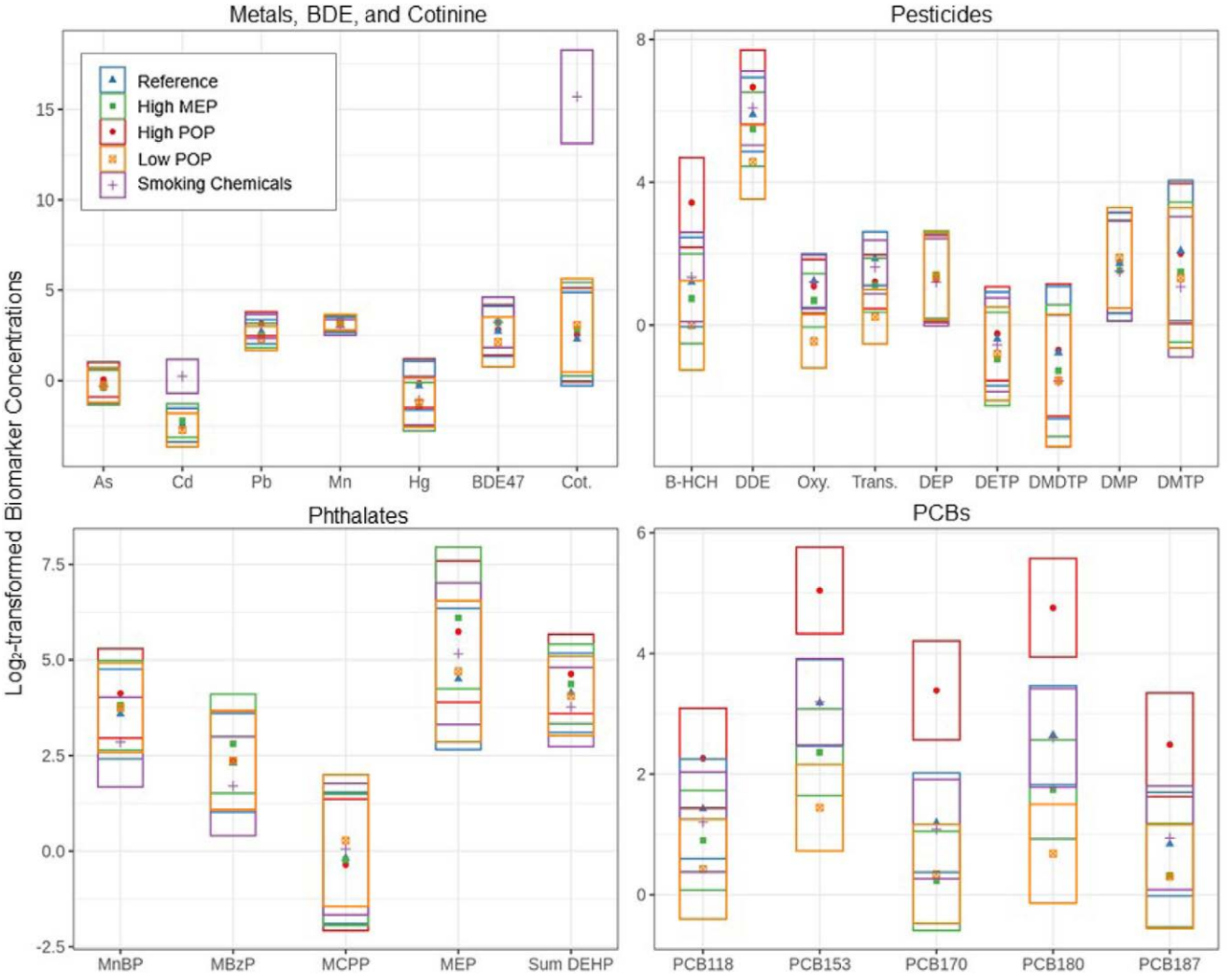

FIGURE 2. Mean $\log_2$-transformed chemical compositions of the five profiles generated by latent profile analysis, separated by chemical group, with boxes showing standard deviation (n = 517). As indicates arsenic; BDE, brominated diphenyl ether; Cd, cadmium; DDE, dichlorodiphenyldichloroethylene; DEP, diethylphosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; Hg, mercury; MBzP, monobenzyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; Mn, manganese; MnBP, monobutyl phthalate; Pb, lead; β-HCH, β-benzene hexachloride.

high), and one with high concentrations of smoking chemicals. However, the cluster counterpart for the low POP profile showed a pattern of higher OPPs and was named the high OPP cluster to reflect this. There were other key differences between the profiles and clusters as well. First, the reference, phthalates, high POP, and high OPP clusters were all similar sizes (n = 115, 133, 111, 140, respectively); in contrast, the smoking chemicals cluster was smaller (n = 18; eTable 5; http://links.lww.com/EDE/B972). This contrasts with the latent profile analysis results, which had much larger reference and high MEP profiles (n = 314 and 136) and much smaller high POP, low POP, and smoking chemicals profiles (n = 30, 19, and 18). Second, the z-scores for the PCB concentrations in the high POP and low POP/high OPP clusters were much closer to zero than in their profile counterparts (Figure 4). Finally, the reference profile had exposure biomarker concentration z-scores that were closer to zero.

When we conducted covariate-adjusted linear regression analysis, we found that the smoking chemicals cluster was negatively associated with all WPPSI-III scores, although 95% confidence intervals crossed the null (FSIQ: –4.1 [–10.5, 2.3]; eFigure 3; http://links.lww.com/EDE/B972). These associations were slightly greater than those found in the smoking chemicals profile and were not sex-specific. The High POP cluster had negative associations with all three scores (FSIQ: –1.4 [–4.8, 2.0]). Unlike in its profile counterpart, there were significant sex-specific associations in this cluster for VIQ (sex interaction: $P = 0.007$) and FSIQ (sex interaction: $P = 0.040$). The High OPP cluster showed sex-specific associations with all three IQ scores; cluster

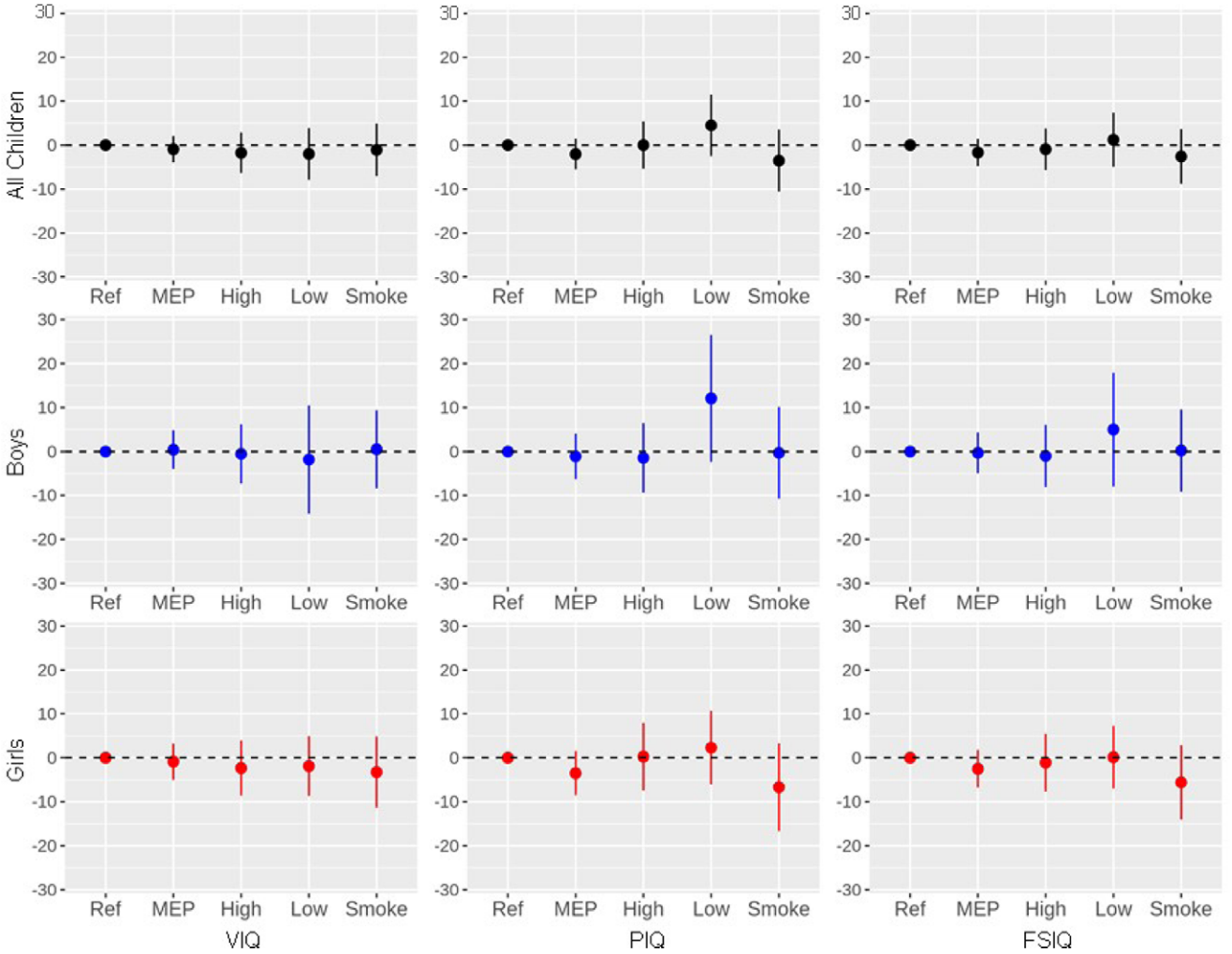

FIGURE 3. Covariate-adjusted linear regression coefficients showing the associations between latent profile membership and WPPSI-III scores, adjusted for maternal age, race, education, and marital status, household income, parity, prenatal alcohol, HOME score, and testing city compared to the Reference Profile, with 95% confidence intervals. Results are shown for all children, and then stratified by sex. We found no significant interactions between latent profiles and child sex (all $P > 0.05$; n = 517).

membership was negatively associated with IQ in boys but had a positive or more weakly negative association with IQ in girls (FSIQ: –1.4 [–6.5, 3.6] and –0.1 [–3.6, 4.7], respectively; sex interaction for VIQ, PIQ, and FSIQ: $P = 0.007$, 0.022, and 0.002, respectively). The phthalate cluster had a weak positive association with VIQ (0.4 [–2.7, 3.5]) and negatively associated with PIQ (–2.2 [5.9, 1.4]) and showing no sex-specific associations.

DISCUSSION

Using latent profile analysis, we generated five latent profiles that showed risk stratification of chemical mixtures in pregnant women: a high MEP profile, a high POP profile, a low POP profile, a smoking chemicals profile, and a reference profile with biomarker concentrations close to zero. We elected to use the medium level profile as a reference for the regression analysis because of its large size, which indicates that this profile may have acted as a catch-all group for mothers who did not fit any of the other patterns.

Overall, when compared to the reference profile, we found negative associations between IQ scores and two of our profiles: the high MEP profile, and smoking chemicals profile, although all 95% confidence intervals spanned the null. However, the associations between the smoking chemicals profile and IQ were mostly found in female children. Although previous studies have found differing effects of neurotoxicants on girls and boys, more research is needed to understand the complex dynamic between gestational smoking, neurodevelopment, and child sex.[1,3,8] The high POP and low POP profiles had differing associations with VIQ and PIQ. The high POP profile had a negative association with VIQ and FSIQ, but neutral associations with PIQ. In the low POP profile, we found the more expected positive association with PIQ and an unexpected negative association with VIQ. Previous studies

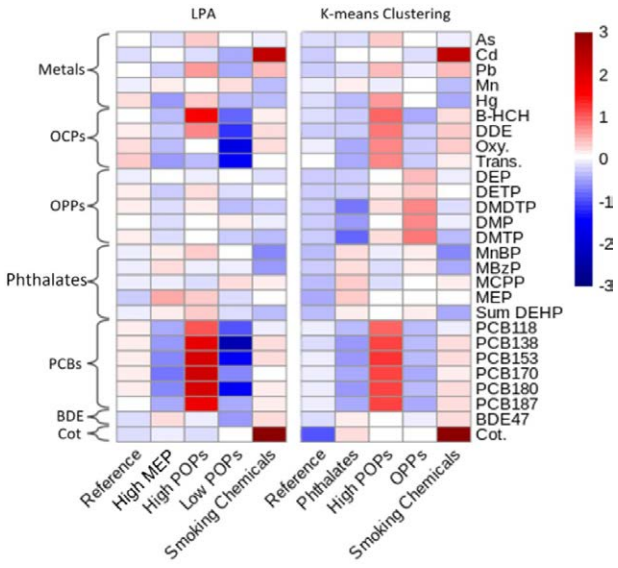

FIGURE 4. Sensitivity analysis comparing latent profile analysis results with k-means clustering results, showing z-scores for the mean biomarker concentrations in each latent profile (left) and k-means cluster (right; n = 517). High concentrations are shown in red, low concentrations in blue. As indicates arsenic; BDE, brominated diphenyl ether; Cd, cadmium; DDE, dichlorodiphenyldichloroethylene; DEP, diethylphosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; Hg, mercury; LPA, latent profile analysis; MBzP, monobenzyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; Mn, manganese; MnBP, monobutyl phthalate; Pb, lead; β-HCH, β-benzene hexachloride.

have shown negative relationships between PCBs and VIQ, but not PIQ, making this result even more surprising.[56,57] However, two noteworthy factors are the size and the sex distribution of this profile; only 19 children most closely matched it, of whom 74% were girls. To solve these problems, further study is needed with a larger population, and more research is needed to determine why differences in associations with PIQ and VIQ can occur.[58–61]

When we conducted sensitivity analysis using k-means clustering, we found that the five clusters had similar patterns to the profiles, albeit less pronounced and with higher levels of OPPs in the low POP cluster. We found that k-means clustering had three main disadvantages: the unclear method of choosing a model, the tendency towards choosing clusters of the same size, and the nonprobabilistic nature of cluster assignment.[29,30] We chose the model with five clusters because that was the number of profiles latent profile analysis generated; however, this would have been a more difficult decision had we not started with latent profile analysis. All but the smoking chemicals cluster were similar in size, which was to be expected given the method's bias towards equal clusters.[29,30] This may have resulted in lower classification accuracy, which we could not easily assess because this method does not generate posterior probabilities. We therefore conclude that, although more research is needed to assess its capabilities, latent profile analysis should be considered for studying the effects of gestational chemical mixtures.

This study builds on other work that has used unsupervised machine learning techniques to estimate the health effects of chemical mixtures. Several similar studies have been done using k-means clustering, which is why we chose this method for sensitivity analysis.[2,25,27,62] Kalloo et al.[2,63] used k-means clustering and PCA to study the effects of chemical mixtures on child IQ. Unlike in our study, they found that the ideal model had three clusters. IQ scores were negatively associated with the clusters with higher concentrations of metals, phthalates, phenols, and pesticides. While these results can not be directly compared with our study, as the mixtures in each cluster are different than those in our profiles, there are some similarities. For example, VIQ and PIQ were not always associated with clusters in the same way; the cluster with the lowest overall biomarker concentrations (cluster 3) had a higher PIQ and a lower VIQ than the cluster with the highest concentrations (cluster 1). We found similarly inconsistent results in our low POP profile.

Another popular technique that has been used to study gestational mixtures is hierarchical clustering, a dimension reduction technique by which chemical exposures can be partitioned into groups.[6,64] For example, in 2020, Mehta et al.[16] used hierarchical clustering and PCA to study associations between gestational exposure mixtures and sociodemographic variables. Like with latent profile analysis, this method does not require one to predetermine the number of groups; however, a key difference is that in this study, the researchers partitioned exposure variables, whereas we were interested in clustering the participants to find associations with chemical mixtures as a whole.

Other studies have also used latent groups created using chemical mixtures. Carroll et al.[23] used latent class analysis, a similar method to latent profile analysis that uses categorical independent variables, to study phthalate and phenols.[65] However, chemical exposures had to be dichotomized for this study. Had we chosen to use latent class analysis, we could not have differentiated the profiles nearly as well, and would have missed information about profiles with moderate biomarker concentrations. In 2018, Hendryx and Luo[24] used latent class analysis to study 47 chemical biomarkers from six chemical groups in children 6–19 years old. They separated their participants into three classes, assigning each participant to a single class based on their posterior probabilities and then regressing class membership against lymphocyte and neutrophil counts. In this study, the number of classes and exposure patterns were different in these groups than in our profiles, perhaps due to the different sociodemographic characteristics in our respective study populations. Finally, a recent study by Khorrami et al.[22] used latent profile analysis to find associations between mixtures of air pollutants and lung cancer. In

this study, however, the researchers used a different combination of chemicals than we did, and pollutant concentrations were ascertained using geographical location, not gestational biomarkers.

Our study has several limitations. The first is that mothers in the MIREC cohort are more likely to be older, wealthier, educated, and White and also have lower rates of self-reported prenatal smoking and alcohol use than the average Canadian who gave birth that year.[32] This trend is found in both the original MIREC study participants and in our study population. Therefore, we may not be accurately reflecting the exposure patterns found in other populations, which limits this study's generalizability. Second, some chemicals had a lower detection rate, and the imputation method we used may have underestimated the variance for these chemicals.[39] Third, exposure misclassification for nonpersistent chemicals may have affected the analysis results.[15] Fourth, because this study was primarily focused on prenatal exposures, we did not account for postnatal factors such as breastfeeding duration, which may have acted as an effect modifier in the associations between chemical exposure and IQ.[28] Fifth, because the information was not available, we were unable to adjust for parental IQ, an important indicator of child IQ. Finally, although the sensitivity analysis with k-means clustering showed approximately the same groups as those of the latent profile analysis within the same sample, no comparison has been made with other samples. If changes were made to the number of participants, the chemical exposures variables, or the study population as a whole, different profiles may be generated. This would also occur if we split our data, especially given the small size of several of our profiles. Our results are informative for studying associations between these specific chemical mixtures and various outcomes, but further study is needed to determine the reproducibility of our profiles. That said, despite these limitations, we believe that latent profile analysis is a promising tool that is worthy of more studies in complex exposure scenarios.

In conclusion, we recommend the use of latent profile analysis as a potential technique for studying chemical mixtures. Although further research is needed to understand the method's capabilities, we believe that this is an effective alternative to other clustering methods. This technique can find patterns in large, complex datasets while avoiding many of the disadvantages of k-means clustering or multiple regression analysis, and it generates a helpful new variable that can be used to study the effects of chemical mixtures on health outcomes.

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