Prenatal Exposure to Endocrine-disrupting Chemicals in Relation to Autism Spectrum Disorder and Intellectual Disability

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Background: Exposure to endocrine disruptors is unavoidable. Many such compounds are suspected to impact neurologic development of children, but most studies conducted have considered effects of individual chemicals in isolation. Because exposures co-occur, it is important to consider their health impacts in a single regression framework.

Methods: We applied Bayesian statistical tools (including shared mean and mixture priors for 25 unique chemicals) to study independent associations of endocrine disruptor biomarkers with autism spectrum disorder (ASD) (n = 491) and intellectual disability (n = 155), compared with 373 general population controls, in the Early Markers for Autism study. We measured biomarkers in maternal serum collected and stored from midpregnancy and considered them individually or as a class (i.e., summed polychlorinated biphenyls). We adjusted all models for original matching factors (child sex and month and year of birth), maternal age, maternal race/ethnicity, parity, and maternal education at the time samples were collected. We estimated the change in the odds of ASD or intellectual disability per 1 SD increase in the z-score of measured biomarker concentration for each chemical.

Results: Odds of ASD and intellectual disability did not change with increasing concentration for any specific endocrine disruptor. The effect estimates for each chemical were centered on or near an odds ratio of 1.00 in both models where we applied a shared mean or a mixture prior.

Conclusion: Our mixtures analyses do not suggest an independent relationship with ASD or intellectual disability with any of the 25 chemicals examined together in this mixtures analysis.

Keywords: Autism spectrum disorder; Bayesian methods; Complex mixtures; Endocrine disruptors

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Humans are regularly exposed to multiple chemicals in day-to-day life. This reality has increased interest in research and methods that consider exposures as mixtures. The term complex mixture broadly refers to any group of exposures that co-occur in time or space or that may share common sources or biologic pathways; a prime example of this is potential endocrine disruptors. Because many of these chemicals can cross the placenta,[1] exposures experienced by pregnant women are of particular concern. Studies examining these chemicals in groups (e.g., summed polychlorinated biphenyls [PCBs] and summed polybrominated diphenyl ethers [PBDEs]) or as individual congeners (e.g., PCB 153 and PBDE 47) suggest that they may impact neurodevelopment.[2–20] However, results of previous research have been inconsistent, suggesting associations, both positive and negative, of specific endocrine disruptors and groups of endocrine disruptors with autism spectrum disorder (ASD)[21,22] and neurodevelopment.[23]

With one exception that we are aware of,[23] the associations of endocrine disruptor exposures to neurodevelopment and ASD have been studied as individual biomarker concentrations or as summed concentrations within chemical classes. This approach overlooks the complex correlations among these chemicals. Further, analyses that do not consider multiple exposures within a single regression framework may be subject to the multiple comparisons problem.[24,25] Historically,

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modeling of one exposure at a time has been favored because of limitations in statistical tools to study multiple agents that occur as a complex mixture. Given the hypothesis that some but perhaps not all endocrine disruptors may play an etiologic role in ASD and other neurodevelopmental disorders, it is important that we apply the most current statistical tools to best understand any potential relationships.

Bayesian methods are ideally suited to study the impact of multiple endocrine disruptors within a single regression model.[24,26] Researchers include quantitative prior information, such as the belief that multiple exposures may have similar effects on a health outcome of interest. Priors may be informed from research in many areas, including epidemiology and toxicology.[27,28] Bayesian methods allow us to use a single regression model to gain insight into the potential influence of multiple, correlated exposures on health while circumventing the multiple comparisons problem.[29]

We applied a Bayesian approach to study the relationship of multiple correlated endocrine disruptor biomarkers to ASD and intellectual disability in the Early Markers for Autism (EMA) study. Our research question concerns whether or not there is evidence of independent associations of endocrine disruptors to ASD and intellectual disability, accounting for co-occurring exposures. These same chemicals were previously studied individually.[21,22,30] We applied Bayesian priors, which assume that effects may be at least partially exchangeable. Results are discussed in the broad context of evidence regarding the potential impact of endocrine disruptor exposure on neurodevelopment.

METHODS

Subjects

The EMA study is a population-based case–control study that was designed to characterize genetic factors and chemical and biologic biomarkers and their relationship to ASD risk.[31] Prenatal and neonatal blood samples were obtained from mother–child pairs as part of the study, which was approved by the institutional review boards (IRBs) of the State of California and Kaiser Permanente Northern California. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research and was thus exempt from IRB approval. Participant mothers were enrolled in California's Expanded Alpha-fetoprotein Prenatal Screening Program between 2000 and 2003 from Orange, San Diego, CA, and Imperial counties, and had given birth between January 2000 and June 2003 in California. Participation in EMA required availability of both maternal prenatal and newborn screening blood samples.

The California Department of Developmental Services (DDS) provides resources and support to individuals of all ages with developmental disabilities including ASD and other pervasive developmental disorders, and intellectual disability, indicated by an IQ <70 based on standardized cognitive and functional tests. Information from DDS records was used to identify children with ASD and children with intellectual disability of unknown etiology (e.g., excluding certain chromosomal defects) who had maternal and newborn blood samples. General population controls were randomly sampled from birth records and matched to ASD (but not intellectual disability) cases on sex, and birth month and year. All children were between 4 and 9 years old at the time of case or control ascertainment.

Trained medical record abstractors conducted diagnostic verification of ASD and intellectual disability. Final case status was determined by expert clinical review of these records based on a published protocol.[32] Classification of ASD was based on Diagnostic and Statistical Manual of Mental Disorders (fourth edition) criteria. Following expert review of records, we reclassified individuals originally identified as intellectually disabled from the DDS system who met ASD criteria as ASD cases. We further evaluated ASD cases for presence of co-occurring intellectual disability. Final case groups include ASD (with or without co-occurring intellectual disability) and intellectual disability (in the absence of ASD).

Maternal Samples

Consent forms obtained from mothers before prenatal screening note that specimens could be used for research purposes on IRB approval. Maternal blood specimens were collected typically between 15 and 20 weeks of gestation (median: 16 weeks, range 7–25 weeks) in serum separator tubes by obstetrical care service providers and underwent alpha-fetoprotein testing within 7 days of collection at a central laboratory (median time = 3 days). Because of their resistance to degradation, chemicals of interest for this work can be measured years after sample collection. After 1–2 days of refrigeration, leftover serum and cell pellet specimens were stored at −20°C.

Quantification of Endocrine-disrupting Chemical Biomarkers

Laboratory methods utilized for quantifying endocrine disruptor biomarkers in EMA maternal serum are described in previous publications.[21,31] Briefly, 37 PCBs, nine organochlorine pesticides (OCPs), eight perfluoroalkyl substance, nine PBDEs, and one polybrominated biphenyls (PBBs) were measured in maternal serum samples at the CDC.[33,34] PCBs, OCPs, PBDEs, and PBBs were measured via gas chromatography isotope dilution high-resolution mass spectrometry (Thermo Fisher Scientific, Bremen, Germany). Samples were processed in batches including unknowns (n = 24), method blanks (n = 3), and quality control (n = 3) samples.[33,35] The limit of detection (LOD) was determined as the highest of (1) the lowest calibration point giving a signal exceeding a signal-to-noise level of 10:1 or (2) three times the standard deviation of method blanks measured in each batch of samples. The median background level if detected in blank samples

was subtracted before assessing whether or not the signal was above or below the limit of detection. The measured concentrations were expressed as picograms per gram of serum (pg/g) and nanograms per gram of serum lipid (ng/g lipid). The limit of detection was calculated for each individual sample and was dependent on the available volume of serum and the serum lipid concentration for expression of the lipid-adjusted LOD. The median volume of serum used was 0.6 g (range: 0.6–1.2 g). A doubling of available serum would reduce the LOD by half. Total cholesterol and triglyceride concentrations were measured using an enzymatic reaction on a Roche ModP analyzer, and the total serum lipid concentration was calculated based on the equations presented in Phillips et al.[36]

We measured eight per- and polyflouroalkyl substances (PFAS) using a modification of a published solid phase extraction–high performance liquid chromatography–isotope dilution tandem mass spectrometry method[34] (current acronyms followed by previously used acronyms as applies in parentheses): perfluorooctane sulfonamide (FOSA/PFOSA), 2-(*N*-ethyl-perfluorooctane sulfonamido) acetate (Et-FOSAA/Et-PFOSA-AcOH), 2-(*N*-methyl-perfluorooctane sulfonamido) acetate (Me-FOSAA/Me-PFOSA-AcOH), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), and perfluorodecanoate (PFDA/PFDeA). For PFAS, concentrations were given in ng/ml, and LODs were 0.08 ng/ml (PFNA), 0.1 ng/ml (PFOSA, Et-FOSAA, PFHxS, and PFOA), and 0.2 ng/ml (PFOS, Me-FOSAA, and PFDA).

Statistical Analyses

The ASD cases in the total study sample included all children who were classified as ASD following expert clinical review: those who were initially ascertained as having ASD (N = 413) and those initially ascertained as having intellectual disability but were then reclassified as having ASD (N = 132). The intellectual disability group includes children classified as having intellectual disability but not having ASD after expert clinical review (N = 181). We restricted our analytic sample to births for which measures of our primary exposures of interest, PCBs, PFASs, PBDEs, PBBs, and OCPs, were available from maternal prenatal samples. After exclusion of individuals with missing exposure information, our total analytic sample included 491 ASD cases, 155 intellectual disability cases, and 373 general population controls.

Of the 64 biomarkers measured, we excluded from analyses those for which >40% of participants had concentrations below the LOD; this was done in prior analyses of these data[21] and has been recommended in previous studies of these pollutants.[37,38] This approach left five PBDEs (PBDE 28, 47, 99, 100, and 153), PBB153, 11 PCBs (PCB 28, 99, 118, 138/158, 153, 170, 180, 187, 194, 196/203, and 199), two OCPs (p,p′-dichlorodiphenyldichloroethylene (DDE), *trans*-nonachlor), and six PFASs (Et-FOSAA, Me-FOSAA, PFHxS, PFOS, PFOA, and PFNA), for a total of 25 biomarkers. For those individuals with a concentration below the LOD for any of the 25 chemical biomarkers considered, we replaced values less than LOD with the LOD/$\sqrt{2}$, as suggested in prior work.[21] We standardized all endocrine disruptors based on their z-score for primary analyses.

Our primary analysis applied a Bayesian approach to study the independent effects of all 25 chemicals within a single unconditional logistic regression model to evaluate the change in the odds of ASD per 1 SD increase in the z-score of measured concentrations for each of the 25 endocrine disruptors. We applied a mixture before the 25 biomarkers using the stick-breaking procedure.[39] Briefly, the mixture prior allows the estimated effect of each biomarker on ASD risk to arise from multiple distributions. In this case, we specified four possible distributions: (1) a positive association between endocrine disruptors and ASD (β_1~N(0.2,1); (2) a negative association between endocrine disruptors and ASD (β_2~N(−0.2,1), 3); and (3) two zero-centered but uninformed normal distributions ($\beta_{3,4}$~N(0,10)). This approach allows us to evaluate the independent effect of each biomarker on ASD risk, but allows for the possibility that these effects are similar. We applied a more commonly used shared mean prior (often referred to as empirical Bayes), which assumes, a priori, that all the biomarkers may share a single, common effect.[24,40] Odds ratios and 95% highest posterior density intervals are reported; we note that the highest posterior density interval is the narrowest part of the posterior density within which some percentage of the distribution lies (here, 95%). We implemented models using the Just Another Gibbs Sampler software (v4.2.0) via the R software package (v3.3.2). We evaluated Gelman–Rubin diagnostics for three different chains to determine model convergence.[41] Models were adjusted for matching factors (child's sex, and month and year of birth), maternal age (continuous), maternal education (less than high school [reference], high school, some college/college degree, and postgraduate), maternal race/ethnicity (non-Hispanic white [reference], Asian, Hispanic, and black/Pacific Islander/other), and parity (1 live birth child [reference] vs. >1 live birth), all obtained from the birth certificate. We also considered inclusion of maternal weight at sample collection as a proxy for body mass index, which was not recorded in the EMA cohort.

In addition to the analyses described above, we conducted secondary analyses of associations with ASD and intellectual disability. We examined alternate parameterizations of the biomarkers. For each of the 25 biomarkers, quartiles based on the distribution of each compound were created to further examine the possibility of associations changing with increasing concentrations of a given biomarker. A Bayesian shared mean prior is applied to each quartile so that the effects of biomarkers are treated as partially exchangeable within the same group. For example, a shared mean is applied to the second quartile of all 25 biomarker concentrations but not to third or fourth quartiles for which a unique prior is applied. We also conducted analyses including only the subgroup of ASD cases

who were ascertained from California DDS as ASD cases before expert clinical review. To examine potential differences by comorbid intellectual disability, we also conducted stratified analyses, examining associations in those diagnosed with both ASD and intellectual disability and in those diagnosed with ASD but not intellectual disability. Finally, we conducted analyses stratified by sex, where differences were qualitatively assessed based on overlap of the posterior distribution.

Statistical software code to recreate these analyses is available as an eSupplement; http://links.lww.com/EDE/B468 and from the corresponding authors' personal web page (http://ghassanbhamra-phd.org/); however, permission to obtain data necessary to recreate these analyses is required (interested parties should contact Lisa Croen).

RESULTS

Table 1 summarizes the distributions of key covariates utilized in these and prior analyses of the association between endocrine disruptor biomarkers and ASD and intellectual disability in the EMA study. We note that results of primary analyses described below were not impacted by the inclusion of maternal weight, so we excluded this confounder from final models. Mothers were of a similar age in ASD and intellectual disability case and general population control groups. Mothers of ASD cases tended to have completed a higher level of education (59% with some college or higher) than controls (47% with some college or higher), whereas mothers of intellectual disability cases were less educated than control mothers (29% with some college or higher). The distribution of maternal race/ethnicity was largely similar for ASD cases and controls. This was not so for intellectual disability cases, whose mothers were disproportionately Hispanic (70%). A lower percentage of intellectual disability cases were male at birth (58%), compared with both ASD cases (82%) and general population controls (83%) because of matching procedures. Finally, mothers of controls (62%) and intellectual disability cases (66%) were more likely to have had prior births than mothers of ASD cases (55%).

TABLE 1. Demographic Characteristics of the EMA Study by Cases–ASD, ID, and GP Control Status

	ASD Cases (N = 545)	ID Cases (N = 181)	GP Controls (N = 418)
Maternal age, mean (SD)	30 (5.6)	27 (6.3)	29 (5.4)
Maternal education, n (%)			
Less than high school	97 (18)	76 (42)	102 (24)
High school	126 (23)	52 (29)	117 (28)
Some college or college degree	222 (41)	42 (23)	143 (34)
Postgraduate education	100 (18)	11 (6)	56 (13)
Maternal race and ethnicity, n (%)			
Non-Hispanic white	192 (35)	32 (18)	138 (33)
Asian	82 (15)	9 (5)	45 (11)
Black, Pacific Islander, and other	48 (9)	13 (7)	35 (8)
Hispanic	218 (40)	126 (70)	197 (47)
Missing	5 (<1)	1 (<1)	3 (<1)
Multiparous, n (%)	298 (55)	119 (66)	259 (62)
Male birth sex, n (%)	446 (82)	104 (58)	345 (83)

GP indicates general population; ID, intellectual disability.

The distribution of serum biomarker concentrations was highly variable (Table 2). The highest concentrations were for p,p′-DDE, as expected. In addition, PBDE47, PCB28, and PFOS had the highest concentrations within their respective chemical class; this was true irrespective of case status. Figure illustrates the correlations across the 25 target biomarkers. Biomarkers within a chemical class were highly correlated and mostly did not correlate with biomarkers in other chemical classes. PBDEs and OCP biomarkers were not strongly correlated within their chemical class as compared with PFASs and PCBs, the latter two being strongly correlated within chemical class.

Table 3 summarizes results from primary analyses of all ASD cases applying a mixture and shared mean prior to the 25 biomarkers under study. There was no evidence of any individual biomarker being associated with ASD when applying a mixture prior; all estimated effects were centered at or near an odds ratio (OR) of 1.00. In analyses utilizing a shared mean instead of a mixture prior, the estimated associations of all 25 biomarkers were close to 1.00.

As with primary analyses, none of the secondary analyses suggested an association between any individual biomarker and ASD or intellectual disability. When examining concentration quartiles for all 25 endocrine-disrupting chemical (EDC) biomarkers, we did not observe a monotonic increase or decrease in the estimated association of biomarkers with either ASD or intellectual disability, nor was there any suggestion of an increasing association among a higher subset of quartiles (such as the fourth vs. the first quartile; eTable 1 [http://links.lww.com/EDE/B468]). All 95% credible intervals greatly overlapped and included an OR of 1.00. When we estimated sex-specific effects with a mixture prior applied to individual biomarkers, there was no suggestion of differences by sex for any of the 25 biomarkers considered; this was true of ASD- and intellectual disability-specific analyses such as those represented in Table 3 (eTable 2; http://links.lww.com/EDE/B468). Results of analyses for continuous concentration, concentration quartiles, and sex-specific continuous concentration effects limited to ASD with comorbid intellectual disability were comparable (in most cases nearly identical) to those where ASD or intellectual disability were independent outcomes of interest (results not shown).

DISCUSSION

Our analysis considered the association of 25 EDCs in a single regression model in a Bayesian framework, an approach that circumvents multiple comparisons problems[29]

TABLE 2. Distribution of EDC Concentrations (ng/g Lipid)[a] in Maternal Serum, EMA Study

	ASD Cases (N = 545)			ID Cases (N = 181)			GP Controls (N = 418)		
Compound	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
PBB153	2.06	1.20	4.80	1.61	0.85	2.13	2.06	1.10	4.57
PBDE28	1.20	0.78	1.48	1.04	0.80	0.83	1.38	0.85	2.06
PBDE47	28.0	13.8	54.6	28.0	14.7	51.6	35.1	16.9	80.0
PBDE99	9.61	4.00	26.2	9.42	4.65	20.5	12.6	4.90	33.6
PBDE100	7.17	3.20	13.6	5.93	3.80	8.18	9.06	3.90	21.4
PBDE153	7.85	3.50	12.6	6.26	3.60	8.11	10.7	4.20	22.4
PCB28	31.5	15.7	39.9	36.0	17.10	45.9	27.3	14.2	35.1
PCB99	1.91	1.50	1.52	1.40	1.17	0.72	1.72	1.30	1.43
PCB118	3.24	2.50	2.84	2.29	1.87	1.52	2.91	2.30	2.46
PCB138_158	8.70	6.65	7.22	5.95	4.45	4.44	7.39	5.50	7.22
PCB153	11.6	9.20	9.38	7.41	5.30	6.00	9.88	7.40	9.78
PCB170	4.09	3.20	3.30	2.75	1.90	2.31	3.54	2.60	3.57
PCB180	10.2	8.00	8.48	6.49	4.40	5.86	8.55	6.10	8.59
PCB187	3.42	2.30	3.51	2.10	1.40	2.03	2.96	1.80	3.92
PCB194	2.48	1.80	2.18	1.60	1.06	1.41	2.14	1.50	2.12
PCB196_203	2.72	2.00	2.35	1.71	1.20	1.42	2.39	1.60	2.43
PCB199	2.64	1.60	2.88	1.51	0.92	1.53	2.25	1.30	2.93
p,p'-DDE	521	207	1190	675	244	1170	674	215	1360
Trans-Nonachlor	6.36	4.90	6.22	5.70	4.45	5.20	5.78	4.70	4.50
Et-FOSAA	0.94	0.70	0.89	1.09	0.80	1.18	1.00	0.70	0.99
Me-FOSAA	1.57	1.20	1.58	1.45	1.10	1.39	1.46	1.10	1.08
PFHxS	2.09	1.30	3.51	2.07	1.30	2.62	1.85	1.30	2.27
PFNA	0.69	0.60	0.38	0.55	0.50	0.32	0.67	0.60	0.40
PFOA	4.25	3.70	2.75	3.92	3.50	2.84	4.19	3.70	2.24
PFOS	20.0	18.1	10.4	18.5	16.9	9.07	20.6	18.3	11.6

Values (and numbers of cases and controls) are representative of the full study sample.
[a]Concentrations are lipid adjusted, with the exception of PFAS (Et-FOSAA, Me-FOSAA, PFHxS, PFNA, PFOA, PFOS), which are presented in ng/ml.

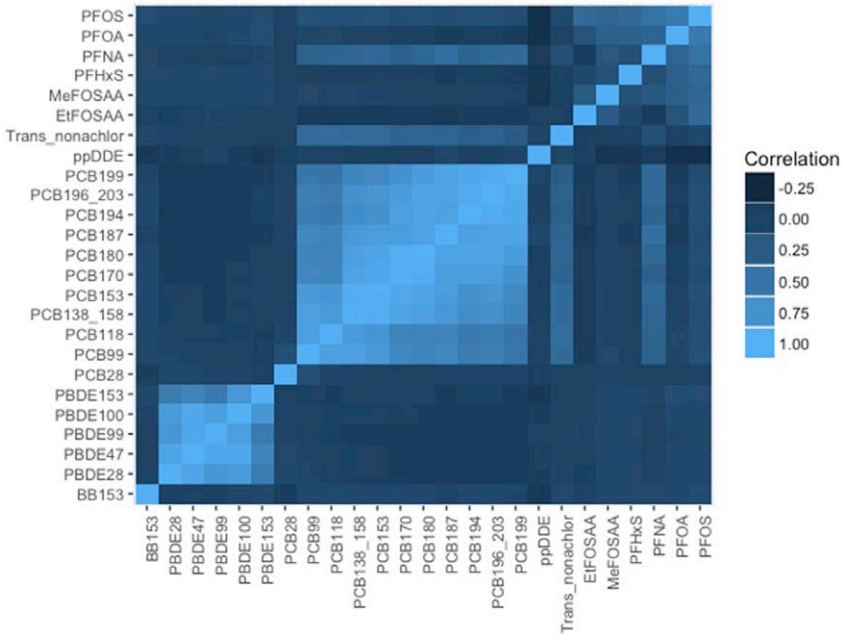

FIGURE. Correlation among endocrine disruptor biomarkers. Please refer to the main text for descriptions of specific chemicals.

TABLE 3. Results of Primary Analyses Comparing ASD Cases (N = 491) and Intellectual Disability Cases (N = 155) with General Population Controls (N = 373)

	ASD		Intellectual Disability	
Chemical	**Mixture Prior OR (95% HPD)**	**Shared Mean OR (95% HPD)**	**Mixture Prior OR (95% HPD)**	**Shared Mean OR (95% HPD)**
PBB153	0.99 (0.96–1.02)	0.99 (0.92–1.05)	0.99 (0.92–1.06)	0.98 (0.83–1.15)
PBDE28	0.99 (0.96–1.03)	0.99 (0.92–1.06)	0.98 (0.89–1.06)	0.96 (0.76–1.14)
PBDE47	0.99 (0.95–1.03)	0.99 (0.91–1.06)	0.98 (0.86–1.07)	0.96 (0.75–1.15)
PBDE99	0.99 (0.96–1.03)	0.99 (0.92–1.06)	0.98 (0.90–1.06)	0.98 (0.80–1.19)
PBDE100	0.99 (0.96–1.03)	0.99 (0.92–1.06)	0.98 (0.83–1.08)	0.96 (0.75–1.17)
PBDE153	0.99 (0.87–1.01)	0.98 (0.88–1.03)	0.98 (0.85–1.06)	0.95 (0.75–1.11)
PCB28	1.00 (0.96–1.04)	1.00 (0.95–1.10)	0.99 (0.92–1.06)	1.00 (0.87–1.20)
PCB99	0.99 (0.96–1.03)	0.99 (0.93–1.07)	0.98 (0.91–1.06)	0.97 (0.78–1.17)
PCB118	0.99 (0.96–1.03)	0.99 (0.93–1.07)	0.98 (0.90–1.06)	0.97 (0.75–1.15)
PCB138_158	0.99 (0.96–1.03)	0.99 (0.93–1.08)	0.99 (0.91–1.10)	1.01 (0.84–1.31)
PCB153	0.99 (0.96–1.03)	0.99 (0.92–1.07)	0.99 (0.91–1.06)	0.98 (0.78–1.21)
PCB170	0.99 (0.96–1.04)	1.00 (0.93–1.08)	0.99 (0.91–1.08)	1.00 (0.82–1.27)
PCB180	0.99 (0.96–1.04)	1.00 (0.93–1.09)	0.99 (0.91–1.08)	0.99 (0.80–1.26)
PCB187	0.99 (0.96–1.03)	0.99 (0.92–1.07)	0.99 (0.91–1.11)	1.00 (0.83–1.31)
PCB194	0.99 (0.96–1.04)	0.99 (0.93–1.08)	0.99 (0.91–1.08)	0.99 (0.81–1.26)
PCB196_203	0.99 (0.96–1.03)	0.99 (0.92–1.07)	0.99 (0.90–1.06)	0.98 (0.76–1.19)
PCB199	0.99 (0.96–1.03)	0.99 (0.93–1.08)	0.99 (0.90–1.06)	0.98 (0.78–1.20)
p,p'-DDE	0.99 (0.92–1.02)	0.98 (0.89–1.03)	0.98 (0.91–1.05)	0.96 (0.79–1.09)
Trans-Nonachlor	0.99 (0.96–1.03)	1.00 (0.94–1.07)	0.99 (0.93–1.25)	1.06 (0.92–1.38)
Et-FOSAA	0.99 (0.95–1.03)	0.99 (0.91–1.05)	0.99 (0.92–1.08)	1.02 (0.90–1.22)
Me-FOSAA	0.99 (0.96–1.04)	1.00 (0.95–1.09)	0.99 (0.92–1.08)	1.01 (0.88–1.24)
PFHxS	1.00 (0.96–1.05)	1.00 (0.95–1.10)	0.99 (0.92–1.21)	1.05 (0.91–1.35)
PFNA	0.99 (0.94–1.03)	0.98 (0.90–1.04)	0.98 (0.90–1.05)	0.96 (0.76–1.11)
PFOA	0.99 (0.96–1.03)	0.99 (0.93–1.06)	0.99 (0.92–1.06)	0.98 (0.83–1.16)
PFOS	0.99 (0.93–1.02)	0.98 (0.89–1.04)	0.99 (0.91–1.05)	0.98 (0.80–1.15)

ORs represent the change in odds per 1 SD change in z-score standardized concentration of a chemical biomarker. Numbers of cases and controls represent those with complete exposure information. All ORs are adjusted for maternal age, education, race, and sex of the child at birth.

HPD indicates highest posterior density.

and overcomes analytic challenges of studying multiple correlated exposures together that hamper traditional statistical tools.[24,26] Overall, our analyses do not suggest an association between any specific endocrine disruptor biomarkers quantified in maternal prenatal serum and the odds of ASD or intellectual disability. Previous analyses have suggested that some of the biomarkers included in the mixture studied here may be related to ASD within the EMA study.[21] These previous analyses utilized single-component regression models to study each biomarker in isolation and summed within their chemical class.[21,22,30] Our Bayesian approach and resultant findings should be balanced against potential limitations of the present analysis and the biologic rationale that justifies this line of inquiry.

Bayesian methods utilize quantitative priors to combine what is assumed or known about effects of exposures with what can be estimated from the data and models used for a given analysis. As demonstrated, researchers can accommodate analyses of multiple, correlated exposure biomarkers within a single regression model.[24,26] Also, the use of a Bayesian approach circumvents the multiple comparisons problem that arises when studying exposure biomarkers one by one and ignoring their correlated nature.[29] These are important benefits for the study of environmental exposures that tend to occur as complex mixtures. We note that analyses here have focused on estimating independent effects of chemicals, rather than attempting to estimate a combined mixture effect, for which other methods are available.[42]

Endocrine disruptors are a quintessential example of exposures that occur as a complex mixture. One study of maternal serum samples taken during the fetal growth period reported at least 25 EDC biomarkers detectable in each sample.[43] The EMA study measured 64 biomarkers in maternal serum collected in midpregnancy, 25 of which were considered here. Figure clearly shows that, within their respective chemical classes, many of the EDC biomarkers detected here are moderately to highly correlated in both positive and negative directions. Nonetheless, most previous analyses of

the relationship of endocrine disruptor exposures to ASD and neurodevelopmental delay have considered effects of each biomarker in isolation or in summed groups. Prior studies have produced inconsistent findings regarding the association between any specific biomarker or summed chemical group and ASD[21,44] or other neurodevelopmental disorders.[45–47] Below, we highlight studies that have considered specific endocrine disruptors and not just summed groups.

In previous EMA analyses assessing individual and groups of endocrine disruptors,[21,22,30] the authors noted increases in the odds of ASD and intellectual disabilities associated with the highest quartile concentrations of PCB 138/158, 153, 170, and 180 (among the PCBs examined). Prior EMA work also observed decreases in odds of ASD and intellectual disabilities in male offspring for those with the highest maternal prenatal concentrations of PBDE 153 and the sum of six PBDEs, as well as different directions in effect for most PBDEs examined for male versus female offspring, which may suggest sexual dimorphism. EMA analyses of PFASs suggested associations below an OR of 1.00. There were modest differences in parameterization of exposures and covariates between prior and current analyses, which are unlikely to explain differences in results. In fact, it is not unexpected that the results here differ from prior analyses by our group. It has been noted previously that suggestions of associations between subsets of correlated exposures can be expected even in the case where there are no true associations. This is the problem of multiple comparisons, noted above.[48] The presence of associations centered at or near an OR of 1.00 for a subset of EDCs present in the model can cause attenuation of effects overall. This is characteristic of the shared mean prior, but only in the case where the data and model are more supportive of exposure effects overlapping; this is, in fact, the case for the previously reported PCB results in EMA, as indicated by overlap of the confidence intervals for individual congeners in those analyses. The mixture prior allows effects of EDCs to differ more easily than the shared mean. Because results based on using either prior remained at or near an OR of 1.00, it may be that prior findings of positive or negative associations for individual chemicals resulted from conducting multiple comparisons on sparse data. Sparsity results from small sample sizes and correlated data, among other reasons.[49]

An analysis of the Health Outcomes and Measures of the Environment (HOME) prospective birth cohort of Cincinnati utilized Bayesian methods similar to those used here to study the relationship between 1 SD increases in 52 EDC biomarkers and neurodevelopmental delay.[23] The authors found a mix of associations across the 52 biomarkers. Our analyses included some of the same biomarkers quantified at the same laboratory, but we did not find supporting evidence of an association with diagnosed ASD or intellectual disabilities. In addition to the HOME and prior EMA analyses, a Danish registry-based study of PFAS found no associations between any specific compounds and ASD diagnosis.[50] Notably, HOME and the national health and nutrition examination survey showed similar patterns and levels of exposure to endocrine disruptors as the EMA study, with the exception of PCB 28 which was more prevalent in the EMA cohort.[23]

Most other prior work has examined summed chemical groups or individual congeners using more traditional statistical approaches. A prior study of summed PCBs suggested a positive association with ASD.[44] Other studies have found evidence of a positive association between specific OCPs and ASD[51] or other neurodevelopmental deficits.[52] A recent systematic review of the literature on PFAS found mixed results: some analyses suggested positive and negative associations of various PFAS with ADHD and neurologic deficit, but most results were consistent with no effects of exposure.[54] In contrast to most previous literature examining these endocrine disruptors, our work found only ORs at or near 1.00.

The biologic rationale supporting a role for endocrine disruptors in adverse neurodevelopmental outcomes is substantial; the US Environmental Protection Agency recognized their importance as early as 1996.[55] In laboratory studies, impacts of endocrine disruptors on normal hormone function have been observed in the nanomolar range; this range is consistent with exposures experienced by humans.[56] Endocrine disruptors may affect neurodevelopment via many mechanistic pathways[20] to impact fetal brain development.[57,58] Notably, gestation is a period of elevated neurologic susceptibility for the fetus.[59–61] Despite a strong biologic rationale for a causal relationship, we did not obtain supporting evidence of an association between specific endocrine disruptors biomarkers with ASD or intellectual disability.

Strengths of this analysis include the following: assessment of exposures in prospectively collected biologic samples during a critical period of neurodevelopment, relatively large sample size, the use of statistical methods to handle a large number of correlated biomarkers and circumvent the multiple comparisons problem, and diagnostic outcomes confirmed by expert clinician review. However, we must acknowledge some potential limitations. First, there is evidence to suggest that time windows of exposure are important to ASD etiology; notable among these are studies suggesting trimester-specific associations.[62,63] Measurements from second trimester samples may differ from other etiologically relevant windows during pregnancy or early postnatal life; however, most of the biomarkers examined here are persistent chemicals, and all have half-lives that would extend through pregnancy, meaning that biomarker concentrations measured in one trimester would correlate highly with and be representative of other trimesters. Second, there is always the possibility of residual confounding. For example, we did not have information regarding prepregnancy body mass index, which is a suspected confounder of the relationship between lipophilic endocrine disruptors and ASD outcomes; however, we considered a range of factors in adjusted analyses, including maternal weight at sample collection, which can be considered a proxy

for BMI. Third, we were limited by analytic sensitivity (i.e., LODs) in our ability to examine a comprehensive set of potential endocrine disruptors. As noted, we only considered 25 of 64 measured biomarkers. If biomarkers of particularly harmful chemicals did not meet the detection frequency inclusion criteria, we would miss their impact on ASD and intellectual disabilities in this analysis. We also did not include other potentially neurodevelopmentally relevant chemical exposures, such as air pollutants, heavy metals, or phthalates;[55,62] thus, our mixtures analysis is not fully comprehensive. Finally, while Bayesian methods are flexible, results may be sensitive to sparse data constraints and the selection of the prior. It is more difficult to detect true effects when the sample size is relatively low and there are a high number of correlated exposures, which result in sparse data challenges.[49] However, our approach allows the information in the data and model to do the bulk of the work by specifying relatively uninformative priors that do not inform the direction or magnitude of the estimated effects.

We previously noted the strength of our Bayesian mixture model.[26] Although we consider biomarker effects as independent, they may be quantitatively similar, a prior belief we leverage in the Bayesian framework. Mixture priors allow shrinkage of effects toward multiple possible distributions. As an example, if a majority of biomarkers does not have an estimable effect and a minority has independent positive or negative effects, then the mixture prior approach allows separation of these effects. There may be concern that exposures with negative correlations and corresponding opposite effects on an outcome will cancel out and shrink toward an estimate of no effect. In fact, the model allows for effects, even those with opposing directions, to be identified so long as there are sufficient data to do so. In our case, all the results were still centered on an OR of 1.00.

In summary, using Bayesian analysis, we did not find evidence of an association between ASD or intellectual disability and 25 individual endocrine disruptor biomarkers. Our results differ from evidence of associations between individual endocrine disruptors and neurodevelopmental outcomes obtained from toxicology research[56] and preliminary evidence from prior ASD and neurodevelopmental epidemiologic studies.[21,23,44,52] Thus, continued exploration of the potential impact of endocrine disruptors, considering combined effects, in association with ASD is warranted.

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REFERENCES

1. Wolff MS, Landrigan PJ. Organochlorine chemicals and children's health. *J Pediatr*. 2002;140:10–13.
2. Eskenazi B, Chevrier J, Rauch SA, et al. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. *Environ Health Perspect*. 2013;121:257–262.
3. Eubig PA, Aguiar A, Schantz SL. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ Health Perspect*. 2010;118:1654–1667.
4. Gascon M, Vrijheid M, Martínez D, et al. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. *Environ Int*. 2011;37:605–611.
5. Herbstman JB, Mall JK. Developmental exposure to polybrominated diphenyl ethers and Neurodevelopment. *Curr Environ Health Rep*. 2014;1:101–112.
6. Jacobson JL, Jacobson SW. Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): the Michigan and North Carolina cohort studies. *Toxicol Ind Health*. 1996;12:435–445.
7. Jacobson JL, Jacobson SW. Prenatal exposure to polychlorinated biphenyls and attention at school age. *J Pediatr*. 2003;143:780–788.
8. Korrick SA, Sagiv SK. Polychlorinated biphenyls, organochlorine pesticides and neurodevelopment. *Curr Opin Pediatr*. 2008;20:198–204.
9. Ribas-Fitó N, Sala M, Kogevinas M, Sunyer J. Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review. *J Epidemiol Community Health*. 2001;55:537–546.
10. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. *Ann Epidemiol*. 1991;1:407–413.
11. Rosas LG, Eskenazi B. Pesticides and child neurodevelopment. *Curr Opin Pediatr*. 2008;20:191–197.
12. Sagiv SK, Kogut K, Gaspar FW, et al. Prenatal and childhood polybrominated diphenyl ether (PBDE) exposure and attention and executive function at 9-12 years of age. *Neurotoxicol Teratol*. 2015;52(Pt B):151–161.
13. Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. *Am J Epidemiol*. 2010;171:593–601.
14. Stewart P, Reihman J, Lonky E, Darvill T, Pagano J. Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance. *Neurotoxicol Teratol*. 2000;22:21–29.
15. Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal PCB exposure and intelligence (IQ) in 9-year-old children. *Environ Health Perspect*. 2008;116:1416–1422.
16. Stewart PW, Reihman J, Lonky EI, Darvill TJ, Pagano J. Cognitive development in preschool children prenatally exposed to PCBs and MeHg. *Neurotoxicol Teratol*. 2003;25:11–22.
17. Canfield RL, Henderson CR Jr, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N Engl J Med*. 2003;348:1517–1526.
18. Axelrad DA, Bellinger DC, Ryan LM, Woodruff TJ. Dose-response relationship of prenatal mercury exposure and IQ: an integrative analysis of epidemiologic data. *Environ Health Perspect*. 2007;115:609–615.
19. Ciesielski T, Weuve J, Bellinger DC, Schwartz J, Lanphear B, Wright RO. Cadmium exposure and neurodevelopmental outcomes in U.S. children. *Environ Health Perspect*. 2012;120:758–763.
20. Schug TT, Blawas AM, Gray K, Heindel JJ, Lawler CP. Elucidating the links between endocrine disruptors and neurodevelopment. *Endocrinology*. 2015;156:1941–1951.
21. Lyall K, Croen LA, Sjödin A, et al. Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability. *Environ Health Perspect*. 2017;125:474–480.
22. Lyall K, Croen LA, Weiss LA, et al. Prenatal serum concentrations of brominated flame retardants and autism spectrum disorder and intellectual disability in the early markers of autism study: a population-based case-control study in California. *Environ Health Perspect*. 2017;125:087023.
23. Braun JM, Kalkbrenner AE, Just AC, et al. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. *Environ Health Perspect*. 2014;122:513–520.
24. Hamra G, MacLehose R, Richardson D. Markov chain Monte Carlo: an introduction for epidemiologists. *Int J Epidemiol*. 2013;42:627–634.

25. Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. *Am J Epidemiol*. 1995;142:904–908.
26. MacLehose RF, Dunson DB, Herring AH, Hoppin JA. Bayesian methods for highly correlated exposure data. *Epidemiology*. 2007;18:199–207.
27. Hamra G, Richardson D, Maclehose R, Wing S. Integrating informative priors from experimental research with Bayesian methods: an example from radiation epidemiology. *Epidemiology*. 2013;24:90–95.
28. Hamra GB, Richardson DB, Dement J, Loomis D. Lung cancer risk associated with regulated and unregulated chrysotile asbestos fibers. *Epidemiology*. 2017;28:275–280.
29. Gelman A, Hill J, Yajima M. Why we (usually) don't have to worry about multiple comparisons. *J Res Edu Eff*. 2012;5:189–211.
30. Lyall K, Yau VM, Hansen R, et al. Prenatal maternal serum concentrations of per- and polyfluoroalkyl substances in association with autism spectrum disorder and intellectual disability. *Environ Health Perspect*. 2018;126:017001.
31. Croen LA, Braunschweig D, Haapanen L, et al. Maternal mid-pregnancy autoantibodies to fetal brain protein: the Early Markers for Autism study. *Biol Psychiatry*. 2008;64:583–588.
32. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. *JAMA*. 2003;289:49–55.
33. Sjödin A, Jones RS, Lapeza CR, Focant JF, McGahee EE 3rd, Patterson DG Jr. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. *Anal Chem*. 2004;76:1921–1927.
34. Kuklenyik Z, Needham LL, Calafat AM. Measurement of 18 perfluorinated organic acids and amides in human serum using on-line solid-phase extraction. *Anal Chem*. 2005;77:6085–6091.
35. Jones R, Edenfield E, Andersen S, Zhang Y, Sjodin A. Semi-automated extraction and cleanup method for measuring persistent organic pollutants in human serum. *Organohalogen Compd*. 2012;74:97–98.
36. Phillips DL, Pirkle JL, Burse VW, Bernert JT Jr, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol*. 1989;18:495–500.
37. Lubin JH, Colt JS, Camann D, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect*. 2004;112:1691–1696.
38. Windham GC, Pinney SM, Voss RW, et al. Brominated flame retardants and other persistent organohalogenated compounds in relation to timing of puberty in a longitudinal study of girls. *Environ Health Perspect*. 2015;123:1046–1052.
39. Lunn D. *The BUGS Book: A Practical Introduction to Bayesian Analysis*. Boca Raton, Fla.: CRC Press, Taylor & Francis Group; 2013.
40. Hamra GB, Loomis D, Dement J. Examining the association of lung cancer and highly correlated fibre size-specific asbestos exposures with a hierarchical Bayesian model. *Occup Environ Med*. 2014;71:353–357.
41. Gelman A, Rubin D. Inference from iterative simulation using multiple sequences. *Stat Sci*. 1992;7:457–511.
42. Hamra GB, Buckley JP. Environmental exposure mixtures: questions and methods to address them. *Curr Epidemiol Rep*. 2018;5:160–165.
43. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ Health Perspect*. 2011;119:878–885.
44. Cheslack-Postava K, Rantakokko PV, Hinkka-Yli-Salomäki S, et al. Maternal serum persistent organic pollutants in the Finnish Prenatal Study of Autism: a pilot study. *Neurotoxicol Teratol*. 2013;38:1–5.
45. Casas M, Nieuwenhuijsen M, Martínez D, et al. Prenatal exposure to PCB-153, p,p'-DDE and birth outcomes in 9000 mother-child pairs: exposure-response relationship and effect modifiers. *Environ Int*. 2015;74:23–31.
46. Gascon M, Valvi D, Forns J, et al. Prenatal exposure to phthalates and neuropsychological development during childhood. *Int J Hyg Environ Health*. 2015;218:550–558.
47. Liu C, Wang C, Yan M, Quan C, Zhou J, Yang K. PCB153 disrupts thyroid hormone homeostasis by affecting its biosynthesis, biotransformation, feedback regulation, and metabolism. *Horm Metab Res*. 2012;44:662–669.
48. Gelman A. Commentary: P values and statistical practice. *Epidemiology*. 2013;24:69–72.
49. Hamra GB, MacLehose RF, Cole SR. Sensitivity analyses for sparse-data problems-using weakly informative Bayesian priors. *Epidemiology*. 2013;24:233–239.
50. Liew Z, Ritz B, von Ehrenstein OS, et al. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. *Environ Health Perspect*. 2015;123:367–373.
51. Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Environ Health Perspect*. 2007;115:1482–1489.
52. Torres-Sánchez L, Schnaas L, Rothenberg SJ, et al. Prenatal p,p´-DDE exposure and neurodevelopment among children 3.5-5 years of age. *Environ Health Perspect*. 2013;121:263–268.
53. Brown AS, Cheslack-Postava K, Rantakokko P, et al. Association of maternal insecticide levels with autism in offspring from a national birth cohort. *Am J Psychiatry*. 0(0):appi.ajp.2018.17101129.
54. Rappazzo KM, Coffman E, Hines EP. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. *Int J Environ Res Public Health*. 2017;14:pii:E691.
55. Kavlock RJ, Daston GP, DeRosa C, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect*. 1996;104(suppl 4):715–740.
56. Vandenberg LN, Colborn T, Hayes TB, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev*. 2012;33:378–455.
57. Pemberton HN, Franklyn JA, Kilby MD. Thyroid hormones and fetal brain development. *Minerva Ginecol*. 2005;57:367–378.
58. Rovet JF. The role of thyroid hormones for brain development and cognitive function. *Endocr Dev*. 2014;26:26–43.
59. Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000;108(suppl 3):511–533.
60. Rodier PM. Developing brain as a target of toxicity. *Environ Health Perspect*. 1995;103(suppl 6):73–76.
61. Mendola P, Selevan SG, Gutter S, Rice D. Environmental factors associated with a spectrum of neurodevelopmental deficits. *Ment Retard Dev Disabil Res Rev*. 2002;8:188–197.
62. Kalkbrenner AE, Windham GC, Serre ML, et al. Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders. *Epidemiology*. 2015;26:30–42.
63. Raz R, Roberts AL, Lyall K, et al. Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' Health Study II Cohort. *Environ Health Perspect*. 2015;123:264–270.