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Chemical mixtures and neurobehavior: A review of epidemiologic findings and future directions

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Abstract

Epidemiological studies have historically focused on single toxicants, or toxic chemicals, and neurodevelopment, even though the interactions of chemicals and nutrients may result in additive, synergistic, antagonistic, or potentiating effects on neurological endpoints. Investigating the impact of environmentally-relevant chemical mixtures, including heavy metals and endocrine disrupting chemicals (EDCs), is more reflective of human exposures and may result in more refined environmental policies to protect the public. In this review, we provide a summary of epidemiological studies that have analyzed chemical mixtures of heavy metals and EDCs and neurobehavior utilizing multi-chemical models, including frequentist and Bayesian methods. Studies investigating chemicals and neurobehavior have the opportunity to not only examine the impact of chemical mixtures, but they can also identify chemicals from a mixture that may play a key role in neurotoxicity, investigate interactive effects, estimate non-linear dose response, and identify potential windows of susceptibility. The examination of neurobehavioral domains is particularly challenging given that traits emerge and change over time and subclinical nuances of neurobehavior are often unrecognized. To date, only a handful of epidemiological studies examining neurodevelopment have utilized multi-pollutant models in the investigation of heavy metals and EDCs. However, these studies were successful in identifying contaminants of importance from the exposure mixtures. Investigators are encouraged to broaden their focus to include more environmentally relevant mixtures of chemicals using advanced statistical approaches, particularly to aid in identifying potential mechanisms underlying associations.

Keywords

chemical mixtures; neurobehavior; multi-pollutant; environmental mixture analysis; statistical methods for exposure mixtures

1. Introduction

Perturbations during highly susceptible periods of neurodevelopment, particularly from the first trimester of pregnancy to the second year of life when rapid brain growth occurs, can profoundly impact child development, with lifelong consequences (1). Early brain development is sensitive to known neurotoxins, including lead (Pb), methylmercury (MeHg), arsenic (As), manganese (Mn), toluene, tetrachloroethylene, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) (2). Other suspected developmental neurotoxins, include cadmium (Cd), bisphenol A (BPA), phthalates, organophosphate pesticides (OPs), fluoride, pyrethroids, triclosan, and perfluoroalkyl substances (PFAS).

The fetus, infant, and child are exposed to mixtures of environmental chemicals from commercial products (pesticides, plasticizers, antimicrobials), air (particulate matter), water (As, PFAS), diet (persistent organic pollutants [POPs], methylmercury), and dust (lead, flame retardants) (3). The staggering majority of epidemiological studies have investigated chemicals in isolation rather than taking into account the totality of chemical exposures, which together may have additive, synergistic, antagonistic, or potentiating effects (4). Findings from epidemiology studies have demonstrated synergistic relationships between environmental exposures and have also reported that nutritional status can interact with chemicals to affect human health. Tobacco smoke has a synergistic relationship with arsenic, asbestos, and radon in inducing lung cancer (5). Folic acid intake has also been shown to attenuate associations between pesticide exposure and autism spectrum disorders in children (6). Synergistic interactions were reported between umbilical cord concentrations of As and nickel (Ni) on the risk of orofacial clefts in a case control study (7). Blood levels of Pb and Cd during late pregnancy had a synergistic relationship on infant neurodevelopment in the Mothers and Children's Environmental Health (MOCEH) Study (8). In the Environment and Childhood (INMA) Project, Freire et al. reported statistically significant synergistic effects between Pb and As on cognition in preschool children, while there was potential antagonistic interactive effects between Mn and Hg (9). Statistically significant interaction between Pb and As was additionally observed in a Bangladeshi cohort, but higher As concentrations were associated with poor neurodevelopmental outcomes in children 2 and 3 years when Pb levels were low (10). In a cross-sectional study of children 8-11 years, blood concentrations of Pb and Mn had an additive interaction on intelligence (11). Given our knowledge of the complex interplay of chemical exposures, there has been a shift in the last decade in environmental epidemiology, where studies are employing advanced statistical models to study the impact of individual chemicals, chemical mixtures, interactions between chemicals, and identifying windows of susceptibility.

Epidemiologic studies of chemical mixtures have typically examined effects of at least two chemicals as an aggregate, either as raw sums, weighted, or molar sums, within generalized linear models (GLMs) with mutual adjustment (4). While GLMs theoretically allow for the identification of important mixture components, coefficient estimates can be sensitive to minor changes in the model specification if multicollinearity is present, limiting its efficiency in identifying important chemicals (4). Examination of chemical interactions in epidemiological studies have relied heavily on the conventional approach of using cross-product terms. However, this approach may be limited by an assumption of linearity in linear models, which may result in identifying synergistic interactions when non-linear interaction dose-response may exist (4). Chemicals may be more toxic during sensitive windows of development; identifying these periods is of great importance to protect the health of populations. The utilization of chemical mixture analyses in identifying periods of heightened susceptibility in children's health were emphasized by Buckley et al., specifying that these advanced methods are able to test period effect differences, address concerns regarding high correlations within exposure mixtures or timing, and integrate repeated measurements of exposures at multiple time periods (12). Further, single-pollutant models may attempt to address confounding by mutually adjusting for all chemical exposures within one model, which could result in inflated or unstable effect estimates. While these chemical mixture approaches have their inherent limitations, their benefit can be greatly enhanced if the selected method is tailored to research questions (13).

Given the limitations of traditional analyses, exploration of more novel and refined methods to address chemical mixtures, including frequentist or Bayesian methods and those involving variable selection, smoothing, dimension reduction, and supervised clustering, have been utilized. However, few epidemiological studies have undertaken advanced statistical models in the investigation of complex mixtures in the detection of neurotoxicological effects. The aims of this review are to summarize studies that have employed multi-chemical models to investigate the impact of chemical mixtures, specifically heavy metals and endocrine disrupting chemicals (EDCs), on neurobehavior, and identify future directions to advance our understanding of how chemical mixtures affect behavioral and cognitive functions in children. This review is intended to encourage and support epidemiologists investigating environmental contaminants and children's neurodevelopment to incorporate multi-pollutant models in their analyses in order to obtain more comprehensive estimates of associations that are consistent with the real-world mixtures of chemicals humans are simultaneously exposed to.

2. Statistical methodologies for analyses of mixtures of heavy metals and EDCs and neurobehavior

Toxicological studies demonstrate that there are chemical interactions in mixtures of heavy metals and EDCs in neurodevelopment. These interactions are mainly based on the two frameworks of independent action and concentration addition. These models differ to that in epidemiology, where interaction is assessed either on the additivity of risk differences or the inclusion of product terms to statistical models (14, 15). However, assessments of interaction in epidemiology are of importance as they can offer some insight on how combined

exposures may affect neurobehavioral outcomes. The development of statistical methodologies to evaluate the potential effect of chemical mixtures has gained considerable traction. Weighted quantile sum (WQS) regression was expanded into lagged WQS to allow for repeated exposure measurements to identify potential windows of heightened susceptibility (16). Time-delayed effects can also be examined using the nonparametric alternative of tree-based distributed lag models (DLMs), based on the random forest algorithm (16). Both WQS regression and tree-based DLMs were applied to the Early Life Exposures in Mexico and Neurotoxicity (ELEMENT) Study to examine prenatal and postnatal exposures to heavy metals and neurobehavior at age 8 years, resulting in the identification of time-varying mixture effects (16). Linear and generalized mixed-effects models (LMM; GMM) with Least Absolute Shrinkage and Selection Operator (LASSO) can also be utilized to identify windows of susceptibility, though no epidemiological study to date has employed this method in the examination of neurodevelopment (17). Schellldorfer et al. (17) released R packages for these Gaussian linear-mixed effects models that will perform a variable selection for high-dimensional covariates, particularly clustered data.

Of the developed models, Bayesian Kernel Machine Regression (BKMR) is able to estimate joint effects, interactions, and allow for nonlinearity using a kernel function (18). It was first utilized to investigate associations between metals in cord blood and psychomotor development among Bangladeshi infants, identifying an inverted U-shaped relationship between Mn and motor composite scores, but only at middle levels of As (18). More recently, Liu et al. developed a flexible method called the Lagged Kernel Machine Regression (LKMR) to identify critical exposure windows, which additionally accounts for non-linear and non-additive effects (19). In the ELEMENT Study, LKMR produced differential time-varying associations for Mn in the analysis of prenatal and postnatal exposures to five heavy metals and visual spatial abilities (19). Mean Field Variational Bayes (MFVB)-LKMR has been developed as a computationally efficient method to analyze exposure to nine metals in three exposure windows in relation to birthweight, although it has not been applied to neurobehavior (20). One of the newer methods is the Bayesian varying coefficient kernel machine regression (BVCKMR), which is a hierarchical model that estimates how chemical mixtures are associated with outcome trajectories (e.g., neurodevelopmental trajectories in children) (21). It accounts for nonlinear and nonadditive effects of individual chemicals and can also assess interactions. BVCKMR has been utilized in the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) Study to examine prenatal levels of nine heavy metals and repeated assessments of cognition from 6 to 24 months (21). Epidemiological studies can substantially benefit from these newly developed approaches to examine chemical mixtures.

3. Environmental epidemiologic studies examining mixtures of heavy metals and EDCs and neurobehavior

Few studies of neurotoxicants have utilized multi-pollutant models in the investigation of chemical mixtures (Table 1) (22-34). Stroustrup et al. (25) examined postnatal exposures among very low birth weight infants to 15 metabolites of 11 phthalate diesters measured in urine within a few weeks after birth and neurobehavioral performance at 33-37 weeks using

WQS regression and noted improved attention, regulation, and social response with increasing phthalate levels. Tanner et al. (34) applied WQS regression in the analysis of 26 EDC exposures during early gestation and IQ in children at age 7 years and reported inverse associations between EDC mixtures and cognitive function in males. They identified bisphenol F as a major contributor in the EDC mixture and several other EDCs of concern, including 3-phenoxybenzoic acid, 3,5,6-trichloro-2-pyridinol, and PFOA. In a cross-sectional study in Uruguay, latent class analysis (LCA) was used to identify four groups of exposure based on postnatal concentrations of metal mixtures (As, Mn, Cd, and Pb) to investigate associations with neurobehavior in children aged 13–42 months (31). Using LCA, they categorized the children's exposure levels as: 1) low metals; 2) low-to-moderate metals; 3) high lead and cadmium; and 4) high metals. However, no relationship was reported between multiple-metal exposure patterns and child neurobehavior.

Frequentist shrinkage methods, including LASSO and elastic net regression (ENR), have been utilized in the Health Outcomes and Measures of the Environment (HOME) Study and the Norwegian Human Milk Study (HUMIS) (24, 28, 30). Zhang et al. (30) observed no differences in the findings between the LASSO analysis and those produced by the traditional single-pollutant approach, reporting prenatal PBDEs were inversely associated with reading skills and full scale IQ (FSIQ) and positively associated with externalizing behaviors at age 8 years. Recently, Guo et al. reported a statistically significant inverse association between prenatal urinary Pb levels and FSIQ at 7 years in the Sheyang Mini Birth Cohort Study (SMBCS) using ENR models (32). In the HUMIS, ENR produced mixed results in the examination of 27 persistent organic pollutants, including PCBs, organochlorine pesticides, brominated flame retardants, and PFAS, measured in breastmilk and clinically diagnosed attention deficit/hyperactivity disorder (ADHD) at 13 years (24). ENR analyses identified positive associations between β -hexachlorocyclohexane (β -HCH) and perfluorooctane sulfonic acid (PFOS) with ADHD diagnoses, whereas there were inverse associations between hexachlorobenzene (HCB) and p,p' -DDT with ADHD diagnoses. In contrast, Forns et al. (28) previously utilized ENR in the analysis of 24 POP mixtures using the HUMIS data and reported that p,p' -DDT was associated with more behavioral problems at 12 months. These authors additionally performed other statistical methods, including principal component analysis (PCA) and the conventional single-pollutant approach utilizing multiple linear regression. All approaches identified positive associations between p,p' -DDT and behavioral problems at 12 and 24 months. PCA also identified an overall positive association between organochlorine pesticide compounds with behavioral problems in infants. Discrepant findings within the HUMIS are likely due to the differences in the outcome of interest (ADHD diagnosis versus the infant toddler symptom checklist [ITSC] score) and the age of assessment.

Bayesian methods are the most frequently employed multi-pollutant methods across environmental epidemiological studies examining neurobehavior. Bayesian methods utilized thus far include Bayesian model averaging (BMA) (28), semi-Bayesian hierarchical regression modeling (29), Bayesian hierarchical modeling (23, 33), Bayesian profile regression (BPR), and BKMR. In addition to ENR and PCA, Forns et al. (28) also used BMA to deal with model uncertainty as inferences are made based on an average of potential models rather than a single 'best' model, with selection of covariates based on the

posterior inclusion probability (PIP). Results from the BMA analysis aligned with findings from the PCA, identifying *p,p'*-DDT and overall organochlorine pesticide compounds as chemicals associated with more behavioral problems in infants. In the SMBCS, Guo et al. additionally utilized BKMR in their examination of a prenatal mixture of 10 heavy metals, pesticides, and phenols and FSIQ in children and noted a significant inverse association between maternal urinary Pb levels and FISQ in boys (32). In the HOME Study, a two-stage semi-Bayesian model was utilized in the examination of prenatal exposure to 52 EDCs and their associations with autistic behaviors at ages 4 and 5 years (29). Findings were mixed, with both positive (BDE-28 and *trans*-nonachlor) and inverse associations (CB-178, β -HCH, BDE-185, PFOA) between various EDCs and parent-completed scores on the Social Responsiveness Scale.

Bayesian hierarchical modeling was used to estimate associations between 23 OP exposures during gestational development and 7 neurobehavioral phenotypes at ages 6-9 years (23). This approach was selected because it addresses collinearity issues between OP metabolites and allows for stable effect estimates to the inclusion of higher numbers of chemicals and potential interactions in the final model. Findings from this study of mother-child dyads within the Mount Sinai Children's Environmental Health Center cohort indicated improved executive function scores with higher dimethylphosphate metabolites, and poorer scores with internalizing behaviors and working memory with increased sum of dimethylphosphate and diethylphosphate metabolites, respectively. Hamra et al. additionally used Bayesian methods in the examination of prenatal exposures to 25 EDCs in relation to diagnoses of autism spectrum disorder (ASD) and intellectual disabilities (ID) in children between 4-9 years in the Early Markers for Autism Study (33). However, no relationship was observed between any of the EDCs and ASD or ID in the mixture analyses.

BPR was used to investigate cluster profiles of 15 pesticides from various chemical classes, including OPs, carbamates, pyrethroids, and neonicotinoids, characterized based on residential address during pregnancy and FSIQ at age 7 years in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) Study (22). BPR identified 8 pesticide use profile clusters. Two of the cluster profiles having the highest cumulative pesticide use levels were associated with decrements in FSIQ when compared to the cluster profile that had the lowest pesticide use level (22). Coker et al. (22) additionally utilized BKMR, the most frequently employed statistical model to examine chemical mixtures and neurobehavior, to identify individual pesticides from the mixtures. BKMR analyses identified oxydemeton-methyl, acephate, and maneb as important contributors to the inverse associations with FSIQ. In a Bangladeshi prospective cohort of 825 mother-child dyads, Valeri et al. (26) employed BKMR to examine the joint effects of cord As, Mn, and Pb levels on cognition in children aged 20-40 months. Findings suggested that there may be potential synergism between As and Mn, with As as a potentiator of Mn toxicity. BKMR was additionally utilized in a cross-sectional study to examine exposure to five metals (As, Mn, Pb, Cd, and selenium [Se]) during adolescence and intelligence following conventional linear regression analyses (27). Results from BKMR were in line with the results of the single-pollutant models, concluding both blood and urinary levels of As as well as Mn, Pb, and Cd were all associated with decreased cognitive scores. However, BKMR identified that

the primary contributors to the inverse association with cognition were blood levels of As and Cd.

4. Biological mechanisms of neurotoxicity among mixtures of heavy metals and EDCs

Toxicological studies provide information on potential pathways of neurotoxicity for heavy metals and EDCs. These neurotoxic mechanisms of action are dependent on the contaminant concentrations and the cell types in the brain. Mechanistic studies have reported biological interactive effects between compounds of heavy metals and EDC mixtures. Since children are not exposed to a single chemical; neurotoxic chemicals may interact with each other and with nutrients to influence brain development in myriad ways.

4.1 Biological mechanisms of neurotoxicity among heavy metal mixtures

Heavy metals share some common pathways for neurotoxicity, including disrupting N-methyl-D-aspartate (NMDA) receptor expression (Pb, As, MeHg), interfering with the Na^+/K^+ -ATPase pump (Cd, MeHg), acting as a competitive ion to Ca^{2+} (Pb, Cd, MeHg), and inhibiting glutamate uptake and clearance (Pb, MeHg), all of which result in an imbalance between reactive oxygen species (ROS) and antioxidant levels (35). Metal mixtures may have greater additive and synergistic neurotoxic responses than the single exposure to one heavy metal. A Pb and Cd mixture had more than an additive effect on divalent metal transporter (DMT1) protein synthesis, resulting in a higher metal transport rate in the cerebellum, cortex, and hippocampus (36). Neurotoxic effects of Pb may further be exacerbated by Mn. Postulated mechanisms include increasing Pb's binding affinity in the brain and jointly interfering with dopamine and neurotransmitter release (37). The ternary mixture of Pb, Cd, and As has been reported to increase neuronal oxidative stress by increasing Ca^{2+} and stimulating the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinases (JNK), and mitogen-activated protein kinase3 (MAPK3) pathways (38). This ternary mixture also had a synergistic effect in augmenting amyloid-beta in the frontal cortex and hippocampus, resulting in cognitive impairments in young rats (39).

4.2 Biological mechanisms of neurotoxicity among EDC mixtures

Possible mechanisms of action for neurotoxicity by EDCs include their ability to mimic thyroid, gonadal, or glucocorticoid hormones, either directly or indirectly via alteration of receptor numbers or affinity to other hormones in the regulation system, which ultimately disrupts endocrine and neurological functions. Other mechanisms of neurotoxicity include their ability to affect transport of hormones as well as cellular metabolism and elimination. *In vitro* studies reported an additive effect on cell viability and oxidative stress in human neuroblastoma SH-SY5Y cells from the binary mixture of BDE-47 and PCB-153 (40, 41). Several studies found that concurrent exposure to several other EDCs, including triclosan, tetrabromobisphenol A (TBBPA), parabens, and di(2-ethylhexyl) phthalate (DEHP), elevated BPA concentrations in mice and consequently the potency of BPA to induce biological effects (42-44). Recent findings reported additive effects of parabens, DEHP, triclosan, and TBBPA, noting magnification of BPA concentrations in the lungs, muscle, uterus, ovaries, kidney, and blood stream of female mice with concomitant exposure to these

other compounds (45). In addition, mixtures of various congeners within one type of EDC may also induce synergistic effects. In a study examining J774A.1 macrophage cells, a ternary mixture of PCB congeners (PCB-101, -153, and -180) resulted in a synergistic effect on apoptosis (46).

4.3 Biological mechanisms of neurotoxicity among mixtures of heavy metals and EDCs

Overall mixtures between suspected and confirmed neurotoxicants outside their general class of compounds may also contribute to neurotoxic insults. In a study of zebrafish, combined exposure to BDE-209 and Pb led to a synergistic and disruptive effect on neurodevelopment, with augmented ROS generation (47). A synergistic relationship was reported in male mice offspring after gestational exposure to BDE-209 and Pb. There was a synergistic increase in pro-inflammation cytokine production in serum and a decrease in the number of neuronal cells in the hippocampus (48). Joint perinatal exposure to MeHg and BDE-99 in rats increased oxidative stress in the cerebellum, whereas exposure to MeHg or BDE-99 alone did not induce oxidative stress (49). Further, there was an increase in BDE-99 concentrations in various brain regions with MeHg co-exposure. Simultaneous exposure to perfluorooctanoic acid (PFOA) and MeHg from gestational day 1 to postnatal day 21 in rats showed that PFOA impaired MeHg retention in various tissues, including cerebellum, cerebral cortex, and hippocampus, suggesting that PFOA was significantly antagonistic to MeHg toxicity (50).

5. Future directions

Evidence from toxicological studies indicate that environmental chemicals not only exert unique mechanisms of neurotoxicity in isolation, but may have much more complex and elusive neurotoxic effects in the presence of other chemicals. Humans are not exposed to a single chemical. As such, traditional methods investigating environmental chemicals in isolation do not fully capture the complete extent of potential effects that xenobiotic agents may have on neurobehavior. That is not to say that single pollutant models are not of importance. Estimating the association between one chemical or one class of chemicals with various neurobehavioral phenotypes have and will continue to yield beneficial knowledge, because they serve as the starting point from which toxicological evidence extends to human health. However, acknowledging and addressing the complexities of the interplay of chemical mixtures will propel environmental epidemiological studies forward. In light of this new period of continued advancements in the development and discovery of multi-pollutant statistical methodologies, epidemiological studies are encouraged be more ambitious in the exploration of chemical mixtures on neurobehavior.

As the availability of multi-pollutant models continues to grow, the selection of the appropriate methodology for chemical mixture analysis and neurobehavior becomes more complicated. Currently there is no gold standard for the analysis of chemical mixtures (4, 51). As such, environmental epidemiologists must select a method that is most appropriate for the study's objectives and research question. We encourage environmental epidemiologists to seek advanced statistical models that can identify important chemicals from the mixtures that are contributing significantly to the association of interest. While it is

important to estimate mixture associations, whether it be a comprehensive mixture reflecting the totality of environmental contaminants, a mixture of one class of exposures (EDCs, heavy metals, OP pesticides), or a mixture based on chemicals by functional use (flame retardants, plasticizers, pesticides), the identification of cumulative effects of multiple chemicals will be more beneficial in improving public health. These findings will provide evidence to support policies to reduce and remove chemicals from the market that are detrimental to neurodevelopment.

Toxicological studies have identified several biological mechanisms of neurotoxicity for chemicals, including interactive effects between binary, ternary, and even quaternary mixtures. For example, increasing levels of Ca^{2+} as well as stimulating the pathways of ERK, JNK, and MAPK3 to increase neuronal oxidative stress has been reported by the ternary mixture of Pb, Cd, and As as a potential mechanisms of neurotoxicity (38). It is important that epidemiological studies assess whether interactions are also present between environmental contaminants in humans, particularly for xenobiotic agents that share a common mechanism for neurotoxicity. These contaminants may have a synergistic relationship that results in more potent neurotoxic effects. This is especially concerning if the synergistic relationship results in perturbations to neurodevelopment at exposure levels that are normally considered to be safe for each individual chemical, but due to the combined presence of both chemicals these low levels are now fully capable of affecting neurobehavior. Identifying nonlinear dose response patterns with chemical mixtures is also a priority. For example, BKMR can be used to assess whether non-monotonicity is present in the associations of chemical mixtures with neurobehavior.

Lastly, investigating critical windows of susceptibility for neurotoxicity (early gestation, mid-gestation, late-gestation, neonatal period, early childhood, mid-childhood, early adolescence) among chemical mixtures is essential to understand differential effects. Timing and dose of chemical exposures may result in various neurobehavioral phenotypes. Critical windows of susceptibility may be different for individual chemicals versus chemical mixtures. Identifying windows of susceptibility for chemical mixtures is possible using LKMR with discrete repeated exposure measurements (19).

6. Challenges in examining chemical mixtures of heavy metals and EDCs and neurobehavior

Epidemiological studies examining chemical mixtures and neurobehavior face unique challenges as neurobehavioral domains vary. Each neurobehavioral domain may have differences in the underlying mechanism(s) of neurotoxic insult by EDCs that may negatively impact development. Further, while many neurobehavior domains are distinct, some are correlated with each other. Second, neurobehavioral development is fluid, with traits that emerge and change over time. In addition, not all neurotoxicants are associated with a clinical diagnosis of a neurobehavioral impairment. Oftentimes the neurotoxicant is associated with decrements in cognitive ability or shifts in behavior frequencies, but at subclinical levels. While they may not be associated with a clinically relevant diagnosis, these shifts in children's neurobehavior are not trivial given the ubiquitous presence of the

chemicals and the impact that it has at the population level. Fourth, a major challenge, not limited to environmental studies examining neurodevelopment, is the availability of environmental contaminant exposure measurements. Multiple measurements are required to identify periods of susceptibility and are recommended for contaminants with short half-lives. This may not be feasible due to limited sample availability and high costs, especially in cohorts of large sample sizes with multiple data collection times. Further, limited studies have examined chemical mixtures and neuroimaging, which may shed some light on the potential mechanisms of neurotoxicity.

7. Statistical challenges of chemical mixture analyses

While statistical approaches for environmental chemical mixtures can address the limitations of the single-pollutant models, there are several concerns that must be considered. First, inclusion of chemical compounds that are highly correlated with each other results in multicollinearity, which may have unknown effects on the directionality and stability of the estimates and inflate standard errors. Multicollinearity can further exacerbate other limitations of chemical mixture models, such as worsening the effects of models that are incorrectly specified as well as increasing confounding by co-exposure collinearity (4, 52). Measurement error of environmental chemicals can have unpredictable effects on the estimates from mixture analyses. Zeger et al. describes the directionality of the bias will be dependent on the strength of the correlation between exposures as well as the extent of the correlation levels and variances in the measurement errors (53). Measurement error can additionally limit the ability to estimate small effect sizes. Third, although many chemical mixture methods have the ability to adjust for potential confounders, some methods, such as partial least squares regression (PLSR) and classification and regression trees (CART), do not provide a straightforward way to do so and require additional steps by the user (54, 55). Fourth, many models require subject-matter expertise to avoid model misspecification; model specifications can affect Bayesian variable selection and subsequently the identification of important chemical exposures within a mixture (4). Lastly, small sample sizes and multiple testing can be problematic.

8. Conclusions

Epidemiological studies stand at the precipice of great change in the examination of chemical mixtures. The development of multi-pollutant models currently exceeds its actual application in neurodevelopmental studies. Several novel methods exist to examine chemical mixtures, but epidemiological studies thus far have not fully taken advantage of all the innovative statistical models. Multi-pollutant models that allow for the identification of important mixture components, estimate mixture associations, assess interactions between environmental contaminants, and identify windows of susceptibility will advance the field of environmental epidemiology. These models are necessary to confirm and strengthen our knowledge of known and potential neurotoxicants in light of the cocktail of chemicals humans are continuously exposed to. While there may be potential discrepancies between studies examining chemical mixtures and neurobehavior that may be attributed to differences in population demographics, cohort-specific confounding effects, chemical exposures measured, exposure patterns, timing of chemical measurements,

neurodevelopmental outcomes, and the selection of statistical methodology, the findings garnered across studies will undoubtedly provide much needed evidence to elucidate the complex nature and impact of chemical mixtures on neurobehavior.

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Environmental epidemiological studies examining mixtures of heavy metals and endocrine disrupting chemicals and neurobehavior

Table 1.

Author (year)	Chemical Mixture			Neurobehavior Assessment		Multi-pollutant Model(s)	Results
	Number	Components	Assessment	Child Age	Battery		
Braun et al. (2014)	52	MBP, MiBP, MEP, MBzP, MCP, MEHP, MECP, BPA, PCB-28, PCB-66, PCB-74, PCB-99, PCB-101, PCB-105, PCB-118, PCB-138/158, PCB-146, PCB-153, PCB-156, PCB-157, PCB-167, PCB-170, PCB-172, PCB-177, PCB-178, PCB-183, PCB-187, PCB-194, PCB-195, PCB-196/203, PCB-199, PCB-206, PCB-209, β -HCH, HCB, oxychlor, <i>trans</i> -nonachlor, BB-153, BDE-28, BDE-47, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, PFOA, PFOS, PFNA, PFHxS	16±3 weeks Maternal Serum Maternal Urine	4 and 5 years	SRS	Two-stage semi-Bayesian model	BDE-28 and <i>trans</i> -nonachlor were associated with more autistic behaviors, while inverse associations were observed between CB-178, β -HCH, BDE-185, and PFOA and SRS scores.
Coker et al. (2017)	15	oxydemeton-methyl, acephate, chlorpyrifos, diazinon, malathion, dimethoate, naled, maneb, methomyl, thiodicarb, permethrin, cypermethrin, cyhalothrin, esfenvalerate, imidacloprid	Residential address during pregnancy	7 years	WISC-IV	BPR BKMR	Of the eight prenatal pesticide profiles identified by BPR, two clusters with the highest cumulative pesticide levels were associated with lower FSQ compared to the cluster with the lowest pesticide level. BKMR identified 3 pesticides from the chemical mixture, including oxydemeton-methyl, acephate, and maneb.
Forns et al. (2016)	24	BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, PCB-74, PCB-99, PCB-105, PCB-114, PCB-118, PCB-138, PCB-153, PCB-156, PCB-157, PCB-170, PCB-180, PCB-189, PCB-194, HCB, β -HCH, <i>p,p'</i> -DDE, <i>p,p'</i> -DDT, oxychlorane	32 days postpartum Breastmilk	12 and 24 months	ITSC	PCA ENR BMA	All models identified positive associations between <i>p,p'</i> -DDT and behavioral problems at age 12 months. ENR and BMA also found positive associations with overall organochlorine pesticides and behavioral problems.
Furlong et al. (2017)	23	DETP, DEDP, DEP, DMTP, DMDP, DMP, MEHP, MEHHP, MEOHP, MECP, MCHP, MEP, MmBP, MiBP, MCP, MBzP, BPA, BP-3, 2,5-DCP, TCS, 3-PBA	25-40 weeks Maternal Urine	6-9 years	BRIEF BASC WPPSI-III WISC-IV	Bayesian hierarchical modeling	DMTP, DMDP, and DMP were associated with worse internalizing behaviors, but improved executive function. DETP, DEDP, and DEP were associated with worse working memory scores.
Guo et al. (2020)	10	Pb, Hg, Cd, 3-PBA, <i>cis</i> -DCCA, <i>trans</i> -DCCA, TCP, BPA, TCS, BP-3	Prior to delivery Maternal Urine	7 years	C-WISC	BKMR, ENR	Prenatal Pb levels were significantly associated with lower FSQ scores in boys at 7 years in both multi-pollutant models. A statistically significant reduction in FSQ score was also observed with higher prenatal Pb levels in both sexes in the ENR model.

Author (year)	Chemical Mixture			Neurobehavior Assessment		Multi- pollutant Model(s)	Results
	Number	Components	Assessment	Child Age	Battery		
Hamra et al. (2019)	25	PBB-153, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, PCB-28, PCB-99, PCB-118, PCB-138/158, PCB-153, PCB-170, PCB-180, PCB-187, PCB-194, PCB-196/203, PCB-199, <i>p,p'</i> -DDE, trans-nonachlor, Et-FOSAA, Me-FOSAA, PFHxS, PFNA, PFOA, PFOS	15-20 weeks Maternal Serum	4-9 years	DSM-IV criteria for ASD and ID	Bayesian hierarchical modeling	No association was observed between prenatal exposure to any of the 25 EDCs in the mixture analysis with either ASD or ID diagnosis in childhood.
Kordas et al. (2015)	4	As, Mn, Cd, Pb	13-42 months Child Urine	13-42 months	BSID-III	Latent class analysis	Null associations were observed between the four groups of metal exposure patterns and neurodevelopment.
Lenters et al. (2019)	27	BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, PFOA, PFOS, PCB-74, PCB-99, PCB-105, PCB-114, PCB-118, PCB-138, PCB-153, PCB-156, PCB-157, PCB-167, PCB-170, PCB-180, PCB-189, PCB-194, HCB, β -HCH, <i>p,p'</i> -DDE, <i>p,p'</i> -DDT, oxychlordane	33 days postpartum Breastmilk Postnatal levels during the first 2 years were estimated via pharmacokinetic model	13 years	ADHD diagnosis	ENR	β -HCH and PFOS concentrations were associated with higher odds of ADHD diagnosis, while lower odds were noted with higher levels of HCB and <i>p,p'</i> -DDT.
Stroustrup et al. (2018)	15	MMP, MEP, MiPP, MnPP, MBzP, MnBP, MiBP, MCP, MOP, MEHP, MEHHP, MEOHPP, MECPP, MCHP, MiNP	Within a few weeks after birth Child Urine	33-37 weeks postmenstrual age (very low birth weight babies)	NNNS	WQS Regression	DEHP metabolites were associated with better performance in attention, regulation, and social response.
Tanner et al. (2019)	26	TCS, BPA, BPF, BPS, MEP, MBP, MBzP, DEHP, DINP, MHDP, MCNP, MOINCH, DPHP, TCP, PBA, 2OHPP, PFOA, PFOS, PFNA, PFDA, PFUnDA, PFHxS, HCB, <i>trans</i> -nonachlor, DDT, ^a PCBs ^b	~10 weeks gestation Maternal urine, serum, plasma	7 years	WISC-IV	WQS Regression	Inverse associations were observed between EDC exposures and IQ in males, with BPF having the largest contribution to the index.
Valeri et al. (2017)	3	As, Mn, Pb	Delivery Cord Blood	20-40 months	BSID-III	BKMR	BKMR revealed a potential synergistic relationship between As and Mn, with As as a potentiator of Mn toxicity.
Wasserman et al. (2018)	5	As, Mn, Pb, Cd, Se	14-16 years Urine, Blood	14-16 years	WISC-IV	BKMR	Decrements in WISC-IV scores were noted with higher levels of As (blood and urine), Mn, Pb, and Cd.
Zhang et al. (2017)	8	BDE-47, BDE-99, BDE-100, BDE-153, PCB-118, PCB-153, PCB-138/158, PCB-180	16±3 weeks Maternal Serum	8 years	BASC-2 WISC-IV WJ-III WRAT-4	LASSO	Prenatal PBDEs were associated with decreased reading skills, decrements in FSIQ, and increased externalizing behaviors in children at 8 years.

Abbreviations: 2,5-DCP, 2,5-dichlorophenol; 2OHPI, 2-hydroxyphenanthrene; 3-PBA, 3-phenylbenzoic acid; ADHD, attention deficit/hyperactivity disorder; As, arsenic; ASD, Autism Spectrum Disorder; β -HCH, hexachlorocyclohexane; BASC, Behavioral Assessment System for Children-2; BKMR, Bayesian Kernel Machine Regression; BMA, Bayesian model averaging; BPR, Bayesian profile regression; BRIEF, Behavior Rating Inventory of Executive Function; BSID-III, Bayley Scales of Infant Development-III; BP-3, benzophenone-3; BPA, bisphenol A; BPF, 4,4-bisphenol F; BPS, bisphenol S; Cd, cadmium; *cis*-DCCA, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acids; C-WISC, Chinese version of Wechsler Intelligence Scale for Children; DEDP, diethylthiophosphate; DEP, diethylphosphate; DEHP, di-(2-ethylhexyl) phthalate; DETP, diethylthiophosphate; DINP, diisononyl phthalate; DMDP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DPHP, diphenylphosphate; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (fourth edition); ENR, elastic net regression; Et-FOSAA, 2-(*N*-ethyl-perfluorooctane sulfonamide) acetate; FSIQ, full scale intelligence quotient; HCB, hexachlorobenzene; Hg, mercury; ID, Intellectual Disability; ITSC, infant toddler symptom checklist; LASSO, Least Absolute Shrinkage and Selection Operator; MBP, monobutyl phthalate; MBzP, monobenzyl phthalate; MCHP, mono(2-carbox-hexyl) phthalate; MCiNP, monocarboxyisononyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; Me-FOSAA, 2-(*N*-methyl-perfluorooctane sulfonamide) acetate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHPP, mono(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MHiDP, monohydroxyisodecyl phthalate; MiBP, mono-iso-butyl phthalate; MiNP, mono-iso-nonyl phthalate; MnBP, mono-*n*-butyl phthalate; MnPP, mono-*n*-pentyl phthalate; MOiNCH, 2,4-methyl-7-oxooctyl-oxycarbonyl-cyclohexane carboxylic acid; MOP, mono-*n*-octyl phthalate; NNNS, NICU Network Neurobehavioral Scale; Pb, lead; PBA, 3-phenylbenzoic acid; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PCA, principal component analysis; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOS, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; *p,p'*-DDE, *p,p'*-dichlorodiphenyldichloroethane; *p,p'*-DDT, *p,p'*-dichlorodiphenyltrichloroethane; Se, selenium; SRS, Social Responsiveness Scale; TCP, 3,5,6-trichloro-2-pyridinol; TCS, triclosan; *trans*-DCCA, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acids; WISC-IV, Wechsler Intelligence Scale for Children-IV; WJ-III, Woodcock-Johnson Tests of Achievement III; WPPSI-III, Wechsler Preschool and Primary Scales of Intelligence-III; WQS regression, weight quantile sum regression; WRAT-4, Wide Range Achievement Test-4.

^a DDT, sum of DDT and its metabolite DDE

^b PCBs: Sum of PCB-74, PCB-99, PCB-118, PCB-138, PCB-153, PCB-156, PCB-170, PCB-180, PCB-183, and PCB-187.