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Chemical Mixtures and Children's Health

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Abstract

Purpose of Review—Humans are routinely exposed to multiple chemicals simultaneously or sequentially. There is evidence that the toxicity of individual chemicals may depend on the presence of other chemicals. Studies on chemical mixtures are limited, however, due to lack of sufficient exposure data, limited statistical power, and difficulty in the interpretation of multi-dimensional interactions. This review summarizes the recent literature examining chemical mixtures and pediatric health outcomes, with an emphasis on metal mixtures.

Recent Findings—Several studies report significant interactions between metals in relation to pediatric health outcomes. Two prospective studies found interactive effects of early life lead and manganese exposures on cognition. In two different cohorts, interactions between lead and cadmium exposures were reported on reproductive hormone levels and on neurodevelopment. Effects of lead exposure on impulsive behavior and cognition were modified by mercury exposure in studies from Canada and Denmark. However, there is little consistency related to exposure indicators and statistical approaches for evaluating interaction.

Summary—Several studies suggest that metals interact to cause health effects that are different from exposure to each metal alone. Despite the nearly infinite number of possible chemical combinations, mixtures research represents real life exposure scenarios and warrants more attention, particularly in the context of uniquely vulnerable children.

Keywords

chemical mixtures; metals; children; pediatric; epidemiology

Introduction

There is a critical need to understand the health effects of chemical mixtures, to protect the public's health. It is well accepted that humans are routinely exposed to multiple chemicals simultaneously or sequentially, and there is evidence that the toxicity of individual chemicals depends on their interactions with other chemicals (1, 2). Evaluating health

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effects of single chemical exposures may underestimate the true effects as in reality everyone is exposed to chemical combinations that may interact. Toxic mixture effects have even been demonstrated when each individual chemical concentration is below its no observable adverse effect level (NOAEL)(3), which is the most realistic exposure scenario for much of the U.S. population. However, research on mixtures remains in its infancy relative to the infinite number of possible exposure combinations.

Metals are a paradigm class of chemicals in which to study mixtures. Metals often co-occur in the environment, in particular among populations living near Superfund sites, in socioeconomically disadvantaged populations, or in urban or industrial regions (4, 5). For example, studies have reported positive correlations between urinary arsenic (As) and blood lead (Pb) levels among subjects living proximal to a copper mine region (6), as well as between blood Pb and blood manganese (Mn) levels in children living in urban environments (7-9). Metals are of particular concern to children's health, due to the relatively high probability of exposure and the ability of metals to individually cause adverse developmental and neurological effects.

A sizable number of experimental studies have assessed interactions between metals. In laboratory animals, co-administration of certain metals has been shown to alter metabolism of individual metals, either by affecting absorption or distribution, compared to administration of each metal separately (10-16). Combined exposure to multiple metals has also been demonstrated to cause greater than additive health effects. Examples include reduced birth weight and brain weight (17); changes in neurotransmitter levels (14, 18, 19); decreased learning (20); and increased spontaneous motor activity (20).

In humans, few studies have been conducted on metal interactions. However, in recent years, the number of published studies has grown, reflecting an expanding emphasis on evaluating health effects of combined metal exposures. It is imperative that clinicians be equipped with knowledge about the implications of combined environmental exposures, so that patients can be advised appropriately. The purpose of this review is to summarize the recent human literature on pediatric health effects associated with exposure to metal mixtures.

Challenges in Chemical Mixtures Research

Although research on the health effects of chemical mixtures spans several decades (21), a number of challenges have limited progress in this field. First, the imprecision inherent in exposure biomarkers, coupled with an uncertainty about which biomarker most accurately represents exposure for each chemical, limits our ability to evaluate chemical mixtures. No single biomarker 'best' represents exposure to all toxicants. Different biomarkers may be needed to accurately represent internal dose of different chemicals, given variations in metabolism and distribution even within the same chemical class. Second, in epidemiologic studies, exposure assessment and knowledge of exposure timing is critical, yet informative exposure data are costly and difficult to collect. Different biomarkers may also reflect different exposure windows, information which is important for selecting an exposure time frame that is relevant for the outcome of interest. In children, the issue of critical

developmental windows comes into play. That is, because children are growing and developing, chemical exposures at specific life stages are often more toxic than similar doses at other ages. Currently, there is a lack of sufficient exposure data on multiple chemicals measured at multiple time points. Third, among studies with adequate exposure data, statistical challenges have limited our ability to analyze and interpret mixture studies (22, 23). Due to the large potential number of predictors and their interactions, there may be insufficient statistical power to detect interactions among exposures. There may also be problems associated with multiple testing, which can result in false positives. Further, multi-dimensional interactions are difficult to interpret, particularly when nonlinear dose-response relationships are present. For example, when, for a given outcome, there exists effect modification of a given metal exposure by levels of a second, correlated metal, it is difficult to differentiate between interaction and a nonlinear exposure-response relationship without additional data. Further, when exposures are highly correlated, results from regression models can become unstable (22). To address these limitations, complex statistical methods have been suggested for estimating effects of multiple pollutants, including supervised clustering methods (e.g., tree-based methods, random forests), Bayesian approaches, dimension reduction approaches (e.g., principal component analysis)(24), and weighted quartile score regression strategies (25). Last, there is a lack of uniform terminology about interaction. Although both toxicology and epidemiology use the word “interaction,” its meaning is understood differently in the two fields. Further, interactions are often described in terms of additive or multiplicative effects of mixture components. There is a lack of data on which form of interaction is more reflective of the true biologic interactions among mixture components. This divergence is a source of confusion in the area of mixtures science (26-28), but an issue that is being addressed with increased communication and collaboration between toxicologists and epidemiologists.

Epidemiologic Studies

We conducted a systematic literature search to identify epidemiologic studies that examined associations of pediatric health outcomes with exposure to more than one metal. We restricted our search to peer-reviewed human studies published in English between January 2010 and December 2013. We located 14 studies that examined effects of combined exposure to multiple metals (Table 1). We summarize results by health outcome: birth defects (29), reproductive outcomes (30), cognitive and motor development (31-39), and behavior (34, 40-42).

Birth Defects

One case-control study examined the association between risk of urinary tract birth defects and heavy metals as a component of a mixture emitted by municipal solid waste incinerators (MSWIs)(29). Exposure to MSWI emissions at maternal residence early in pregnancy was modeled based on data collected from regional MSWIs on constituents including three groups of metals: 1) Pb, Mn, copper, and chromium; 2) nickel and As; and 3) cadmium (Cd) and mercury (Hg). The authors concluded that prenatal exposure to MSWI emissions, which include metals, is associated with increased risk of urinary tract birth defects. However, this approach to exposure estimation requires numerous assumptions; while assumptions are

likely to be non-differential with respect to case status, they can contribute to substantial exposure measurement error.

Reproductive Outcomes

Due to previously reported reproductive and endocrine effects of Pb and Cd separately, Gollenberg et al. (2010) examined associations of reproductive hormones inhibin B and luteinizing hormone with Pb-Cd co-exposure (30). Among 6- to 11-year-old girls who participated in the Third National Health and Nutrition Examination Survey (NHANES III), authors report an interaction between Pb and Cd, whereby the negative association between Pb and inhibin B was stronger in the presence of high Cd. The temporal nature (i.e., which came first) between exposure and outcome cannot be established in this cross-sectional study, but findings are consistent with animal studies of Pb-Cd synergistic effects on hypothalamic-pituitary-adrenal function (43, 44).

Cognitive and Motor Development

Nine studies evaluated metal interactive effects on cognitive and motor development (31-39). Three studies examined interactions between Pb and Mn exposure (31, 33, 34). Two prospective cohort studies evaluated prenatal (33) and early-life (31) metal exposures with early childhood neurodevelopment; both reported significant Pb-Mn interactions. Among two-year-old Taiwanese children with high (>75th percentile) cord blood Pb and Mn levels, significantly lower scores on the Comprehensive Developmental Inventory for Infants and Toddlers (CDIIT) were observed, as compared to children with low cord blood Pb and Mn levels (33). Among 1- to 3-year-old Mexican children, increased Pb toxicity for repeated measures of both mental and motor development was observed among children with high blood Mn levels (highest quintile of exposure), compared to children with lower blood Mn levels (31). In contrast to the aforementioned studies, Lucchini et al. (2012) observed no interactions between blood Pb and either blood, hair, air, or soil Mn in relation to cognitive function, measured using the Wechsler Intelligence Scale for Children (WISC), among 11- to 14-year old Italian adolescents (34). Based on these few studies, it is possible that the Mn-Pb interaction is specific to an early-life developmental window. Synergistic effects between Pb and Mn on neurodevelopment are biologically plausible and may be due to their interactions with similar proteins: Pb disrupts zinc in regulation of NMDA receptor activation, and inhibits Ca²⁺ dependent acetylcholine and dopamine release; Mn also affects dopaminergic and cholinergic neurotransmitters and synaptic modulation, in addition to inhibiting protein transports and enzymes and increasing the release of nitric oxide, involved in cellular signal transduction.

One prospective study evaluated prenatal co-exposure to Pb and Cd and associations with neurodevelopment, assessed using Bayley Scales of Infant Development at 6 months of age (39). Authors reported a potential antagonistic interaction between Pb and Cd in maternal blood during early pregnancy for mental development scores. During late pregnancy, however, authors observed synergistic effects, whereby adverse Pb effects on mental and psychomotor development scores were only significant at high (>median) Cd levels. A synergistic interaction may be related to the ability of Pb and Cd to cause oxidative stress

(45, 46), to interfere with calcium signaling (47), and/or to disrupt neuroendocrine homeostasis such as thyroid hormone (48, 49). The antagonistic interaction between Pb and Cd may be due to a protective effect of Cd at low levels, and/or an interference of Cd with Pb uptake, which has been demonstrated in pregnant mice (50). While these results need to be confirmed in other studies, the shift in direction of the Pb-Cd interaction raises the possibility that mechanisms for interaction between Pb and Cd may depend on stage of pregnancy.

Yorifuji et al. (2011) conducted a prospective cohort study in the Faroe Islands (37). Cord blood Pb and Hg concentrations interacted in their association with cognitive test scores at 7- and 14-years of age. Surprisingly, prenatal Pb exposure was inversely associated with cognition, but only among children in the lowest Hg exposure category, suggesting a less than additive combined effect. One interpretation of this finding is that adverse Pb effects on cognition are masked by the presence of high Hg exposure. Authors hypothesized that Pb and Hg may compete to induce neurotoxic effects.

McDermott et al. (2011) evaluated associations between soil metals at maternal residence during pregnancy and intellectual disability (ID) in ~6- to 11-year-old children (35). Given previous individual associations for Pb and As with ID and the role of hand-to-mouth activity as an exposure route in young children, the authors postulated that combined Pb and As soil concentrations would be associated with elevated risk of ID. They reported a significant Pb-As interaction on ID among normal weight for gestational age infants.

However, the generalizability of these findings is limited to lower socioeconomic status groups, because eligible subjects were those insured by state Medicaid, an insurance plan available to individuals under the federal poverty level. Zahran et al. (2012) also examined soil metal concentrations in relation to school grade point averages using an ecological study design, and reported the lowest grade point averages in schools with the highest total concentrations of metal mixtures in soil (38).

Two cross-sectional studies of 8- to 11-year-old Bangladeshi children examined Mn-As interactions (32, 36). Khan et al. (2011) investigated associations of tube well water Mn and As concentrations, and their interactions, with teacher-reported internalizing and externalizing behaviors (32). They found no evidence of interactions. In a similar study stratified by design on Mn and As concentrations in household wells, Wasserman et al. (2011) found no evidence of Mn-As interactive effects on intelligence (36). Authors noted that only two children in the latter study drank from wells extremely high in both Mn and As, which may partially explain the inability to detect an interactive effect of Mn and As on child intelligence. Exposure levels in Bangladesh may also be so high for both metals that interaction effects are less likely, as each individual metal may have already crossed a threshold of toxicity due to the high exposure levels.

Behavior

Four studies examined behavioral outcomes in relation to multiple metals exposure. Three studies evaluated two-way metal interactions (34, 40, 41) and one evaluated an overall measure of metals exposure (42). None of these studies evaluated interactions between the

same combinations of metals. Boucher et al. (2012) measured Pb, Hg, and PCBs in cord blood and in blood collected between 9 and 13 years of age (40). Effects of prenatal Pb on childhood impulsivity, measured by go/no-go performance, were intensified by higher Hg (and PCB) exposures. In a cross-sectional study, Lucchini et al. (2012) observed no interactions between blood Pb and either blood, hair, air, or soil Mn in relation to the Conners-Wells' Adolescent Self-Report Scale, an assessment of attention deficit/hyperactivity disorder (ADHD)(34). Khan et al (2011) evaluated cross-sectional associations of Mn and As in drinking water with internalizing and externalizing behavior of 8- to 11-year-old Bangladeshi children, but observed no Mn-As interactions (41). Roberts et al. (2013) examined autism spectrum disorder (ASD) in relation to exposure to multiple metals in U.S. EPA-modeled air pollution (42). This nested case-control study identified cases of ASD among offspring of the Nurses' Health Study. Authors noted significant associations of ASD with ambient Pb, Mn, and an overall measure of metals exposure. A proposed mechanism for the link between metals and ASD may be altered thiol metabolism and increased oxidative stress. Heavy metal exposure generates oxidative stress and thiol depletion. Abnormal markers of thiol metabolism, as well as higher levels of hair As and lower levels of hair Hg, copper, and iron were reported in children with ASD compared to age-matched healthy controls (51).

Conclusion

There is a growing body of literature on health effects of chemical mixtures, in particular effects on children. Most metal mixture studies focus on neurodevelopmental outcomes, a common health endpoint for individual metals. Among the reviewed studies, there is little commonality in the combinations of metals evaluated, the exposure indicators selected, and the statistical approaches employed to examine interactions. Despite this heterogeneity, at least one trend is apparent to us: the toxicity of Pb, a well-documented neurotoxicant, seems to increase in the presence of higher levels of other metals, including Mn (31, 33), As (35), Hg (40), and Cd (30, 39).

There are several limitations to the studies reviewed. Most studies assessed only two-way interactions, though effects of higher-order interactions should be considered. In some studies, moderate to high correlations between metals were reported, which can introduce collinearity to statistical models and reduce the reliability of interaction estimates. Few studies considered the possibility of nonlinear interactions between metals, a phenomenon illustrated graphically by McDermott et al. (35). Finally, there is evidence that exposure at different life stages produces different dose-response curves for certain individual metals, such as Pb (52, 53). Very little is known about important developmental windows with respect to metal mixtures exposure, yet only a small number of studies examined interactions at more than one exposure time point (31, 39, 40).

The recent epidemiologic literature suggests that biologic interactions between metals may occur and must be explored further. Regulatory agencies have called for more research on chemical mixtures (21, 54). While a number of challenges have impeded progress in the field of chemical mixtures, there is renewed momentum to address these issues. An

understanding of health impacts of mixed chemical exposure is fundamental for clinicians to advise on exposure reduction strategies.

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Abbreviations

Mn	manganese
Pb	lead
As	arsenic
Hg	mercury
Cd	cadmium

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Key Points

- Humans are routinely exposed to multiple chemicals simultaneously or sequentially, and there is evidence that the toxicity of individual chemicals depends on the presence of other chemicals.
- Metal mixtures are of particular concern to children's health, due to the potential for exposure and the ability of metals to individually cause adverse developmental and neurological effects.
- Though limited, there is some evidence that metals can interact to cause adverse health effects more severe than from exposure to each metal alone. Lead is a metal that is commonly evaluated for its interactive effects and there is suggestive evidence of increased toxicity in the presence of other metals (e.g., manganese, cadmium, mercury, arsenic).
- Several challenges have hampered progress in human studies of mixtures, but interest is growing and new methods are emerging to overcome challenges.

Table 1
Summary of epidemiologic studies examining the association between metal interactions and pediatric health outcomes.

Reference	Study Design	n	Country	Metals Evaluated	Exposure Indicator and Timing of Exposure	Approach for Evaluating Joint Effects / Interaction	Outcome and Age at Time of Assessment	Results
Cordier et al. 2010 (29)	Case-control	304 cases; 226 controls	France	Municipal solid waste incinerator (MSWI) emissions (include heavy metals as component of mixture)	Modeled MSWI emissions during early pregnancy	Assessed as mixture, using dioxin as tracer for mixture	Urinary tract birth defects	Exposure to MSWI emissions in early pregnancy is associated with increased risk of urinary tract birth defects.
Gollenberg et al. 2010 (30)	Cross-sectional	705	United States	Pb, Cd	Pb in blood, Cd in urine, in 6- to 11-year-old girls	Categorized joint exposure: low Pb (<5ug/dl)/high Cd (3rd tertile), high Pb/low Cd, high Pb/high Cd vs. low Pb/low Cd	Reproductive hormones inhibit B and luteinizing hormone (LH), in 6- to 11-year-old girls	Inverse association between Pb and inhibit B was stronger in the context of higher Cd concentrations, suggesting an interaction between Pb and Cd.
Claus Henn et al. 2012 (31)	Prospective cohort	455	Mexico	Mn, Pb	Blood at 1- and 2-years of age	Cross product terms for continuous Pb * categorical Mn (split by highest quintile vs. lower quintiles Mn)	Bayley Scales of Infant Development (BSID-II), from 1- to 3-years of age	Pb toxicity was increased among children with high Mn coexposure, suggesting an interaction between Pb and Mn.
Khan et al. 2012 (32)	Cross-sectional	840	Bangladesh	Mn, As	Drinking water, for 8- to 11-year-olds	Cross product terms	Academic achievement in mathematics and languages, in 8- to 11-year-olds	No Mn-As interactions were observed.
Lin et al. 2013 (33)	Prospective cohort	230	Taiwan	Mn, Pb, As, Hg	Umbilical cord blood	Categorized joint exposure: low Pb (<75th percentile)/high Mn (75th percentile), high Pb/low Mn, high Pb/high Mn vs. low Pb/low Mn	Comprehensive Developmental Inventory for Infants and Toddlers (CDIIT), in 2-year-olds	Co-exposure to high Mn and Pb levels associated with lower scores of neurodevelopment, suggesting an interaction between Pb and Mn.
Lucchini et al. 2012 (34)	Cross-sectional	299	Italy	Pb, Mn	Pb in blood, Mn in blood/hair/air/soil, in 11- to 14-year-olds	Cross product terms for continuous exposure variables	Wechsler Intelligence Scale for Children (WISC) and the Conners-Wells' Adolescent Self-Report Scale Long Form (CASS:L), in 11- to 14-	No interactions were observed between Pb and any measure of Mn exposure.

Reference	Study Design	n	Country	Metals Evaluated	Exposure Indicator and Timing of Exposure	Approach for Evaluating Joint Effects / Interaction	Outcome and Age at Time of Assessment	Results
McDermott et al. 2011 (35)	Retrospective cohort	3988	United States	As, Ba, Cr, Cu, Pb, Mn, Hg, Ni	Soil at maternal residence, during pregnancy	Smooth interaction term between As and Pb (using generalized additive models)	Diagnosis of intellectual disability (ID) in medical records, in ~8- to 12-year-olds	The probability of intellectual disability was significantly associated with the interaction between Pb and As for normal weight for gestational age infants.
Wasserman et al. 2011 (36)	Cross-sectional	299	Bangladesh	As, Mn	As in drinking water/blood/urine, Mn in drinking water/blood, in 8- to 11-year-olds	Cross product terms for continuous exposure variables	Wechsler Intelligence Scale for Children-IV (WISC-IV), in 8- to 11-year-olds	No evidence of As by Mn interactive associations with child intellectual function.
Yorifuji et al. 2011 (37)	Prospective cohort	896	Denmark	Hg, Pb	Hg in cord blood/hair, Pb in cord blood	Cross product terms for continuous exposure variables	Wechsler Intelligence Scale for Children-Revised (WISC-R), Boston Naming Test, and California Verbal Learning Test-Children's version (CVLT-C), at 7 and/or 14 years of age	Hg modified the effects of prenatal Pb exposure within the lowest category of Hg exposure on WISC-R and CVLT-C subtests, suggesting a less than additive combined effect of Hg and Pb.
Zahran et al. 2012 (38)	Ecological	119 schools	United States	Pb, Zn, Cd, Ni, Mn, Cu, Cr, Co, V	Soil	Single index of metals in soil, derived by principal component analysis	4th grade school performance (student scores)	Soil metals accounted for 22-24% of variation in school performance. School grade point averages were lowest where soil metal mixtures were highest.
Kim et al. 2013 (39)	Prospective cohort	884	Korea	Pb, Cd	Mother's blood, during early and/or late pregnancy	Cross product terms for dichotomized exposures, split at median	Bayley Scales of Infant Development (BSID-II), in 6-month-olds	Interactions between prenatal Pb and Cd exposures on neurodevelopment were observed.
Boucher et al. 2012 (40)	Prospective cohort	196	Canada	Pb, Hg	Cord blood; Blood between 9 and 13 years of age (concurrent with outcome assessment)	1) Cross product terms for continuous exposure variables; 2) Continuous exposure variable, stratified by second exposure (split at median)	Go/no-go performance and event-related potentials (ERPs), in 9- to 13-year-olds	Effects of cord Pb were seen primarily in the children with higher prenatal PCB and/or Hg exposures, indicating that the

Reference	Study Design	n	Country	Metals Evaluated	Exposure Indicator and Timing of Exposure	Approach for Evaluating Joint Effects / Interaction	Outcome and Age at Time of Assessment	Results
								effects of prenatal Pb exposure were intensified effects of prenatal Pb exposure were intensified PCB and Hg exposures.
Khan et al. 2011 (41)	Cross-sectional	201	Bangladesh	Mn, As	Drinking water, for 8- to 11- year-olds	Cross product terms for continuous exposure variables	Child Behavior Checklist-Teacher's Report Form (CBCL-TRF), in 8- to 11-year-olds	No Mn-As interactions were observed.
Roberts et al. 2013 (42)	Nested Case-control	325 cases; 22,101 controls	United States	Sb, As, Cd, Cr, Pb, Mn, Hg, Ni, overall metals	U.S. EPA-modeled air pollution, at birth	1) Overall estimate of association with metal exposure using pooled odds ratios estimated for individual metals; 2) Overall measure of metal exposure derived by summing quintile category score for each metal	Autism Spectrum Disorder (ASD)	Perinatal exposure to an overall measure of metals was significantly positively associated with risk of ASD.

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