Review

Inorganic Arsenic Exposure and Children's Neurodevelopment: A Review of the Evidence

David C. Bellinger

Boston Children's Hospital, Harvard School of Public Health, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA; E-Mail: david.bellinger@childrens.harvard.edu; Tel.: +1-617-355-6565; Fax: +1-617-730-0618

Abstract: Experimental studies suggest a myriad of mechanisms by which inorganic arsenic can interfere with central nervous system development, and, indeed, epidemiological studies published in the last dozen years suggest that exposure to arsenic impairs children's cognitive development. Most of the studies have been conducted in developing countries (e.g., Bangladesh, India, Mexico), where exposure to arsenic is thought to be considerably higher than it is in developed countries. This review summarizes the results of these studies, focusing in particular on issues pertinent to risk assessment, including the existence of critical windows of vulnerability, characteristics of the dose-effect relationships (e.g., the lowest adverse effect level, the functional form), the most sensitive neurodevelopmental endpoints, and potential effect modifiers such as host characteristics (e.g., methylation efficiency, sex) and co-exposures to other neurotoxicants (e.g., lead, manganese). At present, the epidemiological data do not permit firm conclusions to be drawn regarding these issues. Several factors that complicate an effort to compare the results of studies are identified, including use of a variety of indices of external and internal exposure, and inconsistency in the measurement of important potential confounders for neurodevelopmental outcomes.

1. Introduction

The adverse effects of inorganic arsenic on health are well-known and include cancer (lung, urinary bladder, skin, and possibly liver, kidney, and prostate), skin lesions (hyperkeratosis, pigmentation changes), lung disease (pulmonary interstitial fibrosis), and cardiovascular disease [1–3]. It is only recently, however, that arsenic's potential as a developmental neurotoxicant has been considered.

The primary route of exposure to arsenic in most settings is consumption of contaminated drinking water [4] and food [5], particularly rice [6], even in areas such as the U.S. [7]. Arsenic crosses the placenta (though the mammary gland to only a limited extent) and exposure typically occurs from the beginning of life. Indeed, greater levels of prenatal exposure have been associated with a variety of adverse pregnancy outcomes, including fetal loss (spontaneous abortion) and infant mortality [7–9], reduced fetal size [5,10], and reduced growth velocity [10]. Increased prenatal exposure has also been associated with reduced duration of gestation [4,8] and increased risk of birth defects [11].

The diverse potential mechanisms of arsenic neurotoxicity lend biological plausibility to suspicion that early exposure perturbs the development of the central nervous system. These mechanisms, identified in experimental animal studies, include oxidative stress and the production of free radicals resulting in neuronal apoptosis [12,13], impaired hippocampal neurogenesis [14], dysregulation of the hypothalamo-pituitary-adrenal axis including reduced levels of corticosterone receptors in the hippocampus [15,16], epigenetic effects such as reduced DNA methylation in the hippocampus and frontal cortex [17], reductions in brain levels of biogenic amines and other neurotransmitters [18–21], changes in the expression of the NMDA receptor complex [22], inhibition of neurite outgrowth [23], structural malformation of white matter (e.g., myelin sheaths) [24] and of hippocampal mossy fibers [25], and endocrine disruption, including down-regulation of thyroid hormone receptor genes [26,27].

An episode of clinical arsenic poisoning that occurred in Japan in 1955 provides a proof-of-concept demonstration that high-dose arsenic exposure affects children's neurodevelopment. In this event, tens of thousands of children were poisoned as a result of the contamination of one manufacturer's milk powder with disodium phosphate that contained 5%–8% arsenic, resulting in a concentration of 20–30 mg/kg in dried milk and a concentration of 5 mg/L in prepared milk [28]. An infant's daily arsenic intake was estimated to be 3–5 mg, and signs of poisoning appeared after a total intake of approximately 60 mg of arsenic. Infants presented with anorexia, diarrhea, vomiting, abdominal distention, fever, and abnormal skin pigmentation. There were 130 certified fatalities, but as many as 25% of infants born in the 14 prefectures of western Japan were affected. Post-mortem examination revealed brain edema, cerebellar hemorrhage, and myelin degeneration, and follow-up studies of survivors identified high rates of epilepsy, severe mental retardation (IQ < 50), and hearing disability.

This review focuses on studies that investigated whether arsenic exposures lower than those responsible for the milk powder poisoning episode, and, indeed, below those those associated with changes in skin pigmentation, produce milder forms of neurodevelopmental dysfunction, as measured by endpoints such as neonatal behavior, intelligence, neuropsychological function, and behavior. This is a relatively new field of research, as the first epidemiological studies of low-level arsenic exposure were published only at the turn of the 21st century. A systematic review was conducted using the search term "arsenic" combined with "brain" (490 papers), "intelligence" (30 papers),

"child development" (72 papers), "cognition" (21 papers), and "child behavior" (16 papers). Abstracts were reviewed to identify empirical studies that reported the assessment of arsenic exposure (either water concentration or biomarker levels) and children's neurodevelopment. The key methodological features of studies identified as relevant, including study site, sample size, age of children, and exposure metric(s), are presented in Table 1. The review is organized around questions germane to a risk assessment of pediatric arsenic neurotoxicity, including the following:

- Are there critical windows of vulnerability, *i.e.*, do the nature and severity of the impacts of arsenic depend on the developmental stage at which exposure occurs?
- What is the dose-effect relationship for neurodevelopmental endpoints, *i.e.*, the lowest adverse effect level, the functional form of the relationship, and the severity of the exposure-related deficits?
- What are the most sensitive neurodevelopmental endpoints?
- Are there effect modifiers, *i.e.*, does the dose-effect relationship depend on host characteristics (e.g., sex, methylation efficiency) or co-exposures to other neurotoxicants?
- What are issues pertinent to modeling arsenic's effects on neurodevelopment, *i.e.*, what variables are critical for future studies to measure and incorporate into analytic models?

Table 1. Major epidemiological studies of neurodevelopmental toxicity of arsenic.

Ref no.	Publication year	Site	*N*	Age	Exposure
[29]	2001	Mexico	80	6–9 years	UAs:Exposed group: 62.9 ± 0.03 μg/g Cr (range:27.5–186.2) Reference group: 40.2 ± 0.03 μg/g Cr (range: 18.2–70.8)
[30]	2003	Taiwan	49	13 years	Water As:High exposure group: 185.0 ± 225.9 μg/L Low exposure group: 131.2 ± 343.7 μg/L
[31]	2004	Bangladesh	201	10 years	Water As: 177.8 ± 145.2 μg/L (range: 0.094–790) UAs: 296.6 ± 277.2 μg/g Cr
[32]	2006	USA	31	12–13 years	Hair As: 17.8 ± 14.1 μg/L (1.4–55.4)
[33]	2007	Bangladesh	301	6 years	Water As: 120.1 ± 134.4 μg/L (range: 0.10–864) UAs: 347.7 ± 352.7 μg/g Cr
[34]	2007	India	351	5–15 years	Water As:Peak lifetime: 147 ± 322 μg/L (range: 1–2480) Average lifetime: 59 ± 133 μg/L (range: 1–870) Pregnancy: 110 ± 243 μg/L (range: 1–2536) Child UAs: 78 ± 61 μg/L (range: 2–375)

Table 1. *Cont.*

Ref no.	Publication Year	Site	*N*	Age	Exposure
[35]	2007	China	720	8–12 years	Water As:High exposure group: 190 ± 183 μg/L (range:14–502) Medium Exposure group: 142 ± 106 μg/L(range: 7–303) Control group: 2 ± 3 μg/L (range: 1–10) UAs:High exposure group: 73 ± 3 μg/L (range:17–595) Medium exposure group: 46 ± 3 μg/L (range: 9–315) Control group: 10 ± 2 μg/L (range: 3–47)
[36]	2007	Mexico	602	6–8 years	UAs: 58.1 ± 33.2 μg/L (52.3% > 50; 9.8% > 100)
[37]	2009	Bangladesh	1799	7 months	Maternal pregnancy urine:GW 8: median 81 μg/L (IQR: 37–207) GW 30: median 84 μg/L (IQR: 42–230)
[38]	2010	Bangladesh	2112	18 months	Maternal pregnancy urine:Mean of GW8 and GW30: 96.3 μg/L (IQR:46–219) Child urinary As at 18 months: 34.6 μg/L (IQR: 18–80.2)
[39]	2011	Bangladesh	~1700	5 years	Maternal pregnancy urine As:GW8: median 81 μg/L (10th: 24, 90th: 380) GW30: median 84 μg/L (10th: 26, 90th: 415) Child urinary As:1.5 years: median 34 μg/L (IQR: 12, 155) 5 years: median 51 μg/L (IQR: 20,238)
[40]	2011	Bangladesh	299	8–11 years	Water As: 43.3 ± 73.65 μg/L Child UAs: 78.1 ± 72.2 μg/L Child Blood As: 4.8 ± 3.2 μg/L
[41]	2011	Mexico	526	6–7 years	UAs: median 55.2 μg/L (IQR: 39.7; range: 7.7–215.9)
[42]	2011	Nepal	100	1 day	Cord blood As: median 1.33 (range: 0.51–9.58)

Abbreviations: UAs: urinary arsenic; Cr: creatinine; IQR: interquartile range; GW: gestational week.

Before the results of individual studies are discussed, several general considerations are addressed. Any attempt to integrate the findings of studies for the purpose of addressing the risk assessment questions posed above is potentially complicated by the fact that the studies differ in certain critical respects. Most have been conducted in developing countries where it is known that exposures to

arsenic are, on average, relatively high due to the local geology (Bangladesh, India, Taiwan, Nepal, China) or because of the presence of industrial point sources (Mexico). Depending on the functional form of the dose-response/effect relationship (*i.e.*, linear, nonlinear), the effect estimates might not be directly comparable across studies. On the other hand, even in studies conducted in areas considered to have "high" arsenic exposure, considerable variability is evident in water arsenic and biomarker levels, overlapping with the distributions of these values in areas considered to have "low" exposures. Of greater concern are the substantial differences in the numbers of children contributing data to each study, with sample sizes ranging from 31 to more than 2000. Also, the ages of children in the different cohorts range from newborn to 15 years. The neurodevelopmental outcomes that can be assessed in children across this age range differ considerably, both in terms of the domains and their potential sensitivity to inorganic arsenic exposure.

Study sites also differ somewhat in terms of the distributions of certain potential effect modifiers, such as malnutrition and co-exposures to other neurotoxicants. For instance, if increased oxidative stress is one of the mechanisms of arsenic neurotoxicity, the effects would be expected to be greater among children with poor nutritional status, specifically low dietary intake of anti-oxidants. Similarly, children with low micronutrient status and protein intake might show greater adverse effects because these deficiencies reduce the efficiency of arsenic methylation. Unless such factors are addressed in analytic models, the dose-effect relationships might not be comparable across studies. On the other hand, observing similar, significant inverse relationships between arsenic exposure and neurodevelopment in study cohorts that differ in these factors might, as was the case with lead [43], provide important data regarding the likely validity of a causal relationship.

In addition to inter-study differences in the ranges of exposure represented in a cohort, the exposure indices measured differ across studies. Some studies are ecologic in design, using measures of arsenic exposure that pertain to an area (e.g., town) rather than measures that provide information about the exposure of individual children. Among studies that measured individual exposures, some relied on biomarkers such as blood, urinary, and hair arsenic, while other studies measured only external exposures such as water arsenic concentrations at different time points (e.g., concurrent, peak, gestational, lifetime). In studies in which both external and internal exposures were measured, the correlations between current water arsenic and urinary arsenic concentration were often modest, e.g., 0.12 [34], 0.31 [33], and 0.45 [31], suggesting that the information they provide about exposure is complementary rather than redundant. Higher correlations tend to be found between different arsenic biomarkers (e.g., pregnancy urinary and blood arsenic) [44]. This is likely due, in part, to the fact that indices of internal exposure, such as urinary or blood arsenic, reflect exposure from all sources including food. Interpretation of water arsenic levels as a proxy index of exposure is complicated by differences over time (e.g., season) in water consumption and the fact that an individual tends to consume water from several sources that might differ in arsenic concentration. The exposure metrics used in different studies also vary in terms of the developmental window sampled. In most studies, exposures at specific time points (e.g., early gestation, late gestation, childhood) were used in analyses, but in some (e.g., [30]), lifetime exposure was calculated (*i.e.*, arsenic concentration in water X drink years X 365 days X volume of water consumed).

In studies that relied on urinary arsenic concentration as the exposure index, some adjusted for creatinine (e.g., [29,31,33,40]), which is highly age-dependent, (or specific gravity) while others did

not. Roy *et al.* [41] argued that adjustment is not appropriate because creatine, a creatinine precursor, is linked to one-carbon metabolism and is therefore a predictor of arsenic methylation. There is no consensus, as yet, as to how to address this concern.

Studies also differ in terms of the instruments used to assess neurodevelopment. In many cases, tests developed and normed on children in the United States were adapted and, as a result, analyzed as raw rather than standardized scores due to uncertainty about the validity of the US norms.

2. Critical Windows of Vulnerability

One obstacle to drawing inferences about the relative importance of children's exposures during different developmental windows is the fact that relatively few studies collected data using multiple metrics that reflect different exposure averaging times. In addition, some studies measured biomarker levels at some time points (e.g., urinary arsenic), but external exposures (e.g., water arsenic level) at others, impeding direct comparison of their associations with health endpoints.

Some studies have reported that children's neurodevelopment is associated with maternal exposure during pregnancy. For instance, in a study using a Baysian Kriging model, arsenic concentrations in the soil to which a woman was exposed during pregnancy [45], particularly the first trimester [46], predicted her child's risk of later intellectual disability. In the few studies that collected data on arsenic exposures during different periods of a child's life, it appears that biomarkers of recent exposure might be somewhat more strongly associated with neurodevelopmental test scores than are biomarkers of prenatal exposure. In a study conducted in West Bengal (India) [34], test scores at ages 5–15 years were inversely associated with children's urinary arsenic concentrations but not with peak arsenic concentration in the water consumed by the mother during pregnancy, or with cumulative water arsenic exposure in the interval since birth. In a prospective study in Bangladesh [37–39], IQ score at age 5 years was inversely associated with mothers' urinary arsenic concentrations at weeks 8 and 30 of gestation and with children's urinary arsenic at 18 months, but the associations were strongest for children's urinary arsenic at the time of testing. In two cross-sectional studies conducted in Bangladesh, IQ scores at age 6 [33] and 10 years [31] were inversely associated with current water arsenic levels (but not with children's urinary arsenic levels). Although these studies were conducted on different cohorts of children, the fact that the associations were stronger in the study of 10 year olds than in the study of 6 year olds was interpreted as suggesting the importance of exposure duration rather than critical windows. Other data consistent with this hypothesis is the finding from the Bangladeshi prospective study that it was only neurodevelopment at 5 years, and not at 7 and 18 months (Bayley Scales of Infant Development, Problem-Solving Test), that was associated with arsenic exposure [37,38]. On the other hand, this finding might have a methodological explanation, as children's urinary arsenic level at 5 years was approximately 50% higher than it was at 18 months of age, and neurodevelopmental testing at 5 years of age is generally more reliable than is testing at 7 or 18 months of age.

One factor that has been proposed as an explanation for reduced vulnerability of children during gestation is the increase in arsenic methylation efficiency that occurs early in pregnancy [44]. However, it seems premature to draw firm conclusions at this time about age-dependent differences in children's vulnerability to arsenic neurotoxicity, much less about potential mechanisms.

3. Dose-Effect/Response Relationships

Important inputs to an arsenic risk assessment would be reliable information about the lowest-observed adverse effect level and the functional form of the association between exposure and adverse neurodevelopmental outcomes. The major obstacle to achieving such knowledge is the diversity of measures of internal and external exposure to which neurodevelopment has been related. As noted earlier, the exposure metrics used in different studies might not provide information about the intervals, so one would not necessarily expect the dose-effect relationships reported in the studies to be the same. To make progress on this issue, it will be important for studies to measure a variety of exposure metrics at different developmental periods in order to permit comparisons among them in terms of the relative strength of their associations with neurodevelopment.

The clearest evidence available on the dose-effect relationship between water arsenic level and IQ is provided by the Bangladeshi study of Wasserman *et al.* [31], in which dose-related decreases in raw Full-Scale and Performance IQ scores and, to a lesser extent, Verbal IQ in 201 10-year olds (adjusted for maternal education, maternal intelligence, type of housing, child height, child head circumference, and access to television), were observed across quartiles of water arsenic (0–5.5 µg/L, 5.6–50.0, 50.1–176, 177–790). The association between IQ score and water arsenic concentration was curvilinear, with a greater decline in IQ per µg/L of arsenic at lower than at higher concentrations. This could be an artifact of the log transformation applied to water arsenic and which forced such a form on the relationship. However, the decline in children's IQ scores appeared to be roughly linear across quartiles and, given the substantial positive skew in water arsenic levels, this is consistent with a supra-linear relationship. In contrast, in a small study that evaluated the association between hair arsenic and Verbal IQ among 12–13 year olds [32], the inverse association observed appeared to be linear. These findings are not necessarily incompatible, however, as different exposure metrics were used, and it is likely that different ranges of arsenic exposure were represented in the two study cohorts.

The diversity of the ways in which estimates of effect size are reported in different studies also contributes to the difficulty of comparing these estimates. Expressing water arsenic concentration as a log-transformed continuous variable, Wasserman *et al.* [31] reported that raw Full-Scale IQ declined 3.8 points as concentration increased from 0 to 10 µg/L, and an additional 2.6 points as it increased from 10 to 50 µg/L. In the study of Hamadani *et al.* [39], a 100 µg/L increase in concurrent (age 5 years) urinary arsenic level was associated with a 1–3 point decline in IQ (over a urinary arsenic range of 20–238, with a median of 51). In a meta-analysis, Rodriguez-Barranco *et al.* [47] reported that a 50% increase in urinary arsenic was associated with a decline in IQ corresponding to 40% of a standard deviation (~6 points) in children 5–15 years of age. In a meta-analysis of four ecologic Chinese studies, the weighted mean deficit in the IQ scores of children living in the "arsenicosis" towns, compared to the children living in the "non-arsenicosis" towns, was 6.85 points [48]. In the Bengali study of von Ehrenstein *et al.* [34], children in the upper tertile of urinary arsenic (>83 µg/L) had reductions of 12%–24% in their scores on selected IQ subtests. In two studies conducted in Bangladesh, water arsenic level accounted for approximately 4% of the variance in the IQ scores of 10 year olds [31] and approximately 1% of the variance in the IQ scores of 6 year olds [33]. In a study conducted in China, the frequency of IQ scores $\leq$ 70, the cut-off generally used to identify children

with mild intellectual disability, was 8.3% in the group with "high" water arsenic levels (mean of 192 µg/L), 3.3% in the group with "medium" water arsenic levels (mean 142 µg/L), and 0% in the group with "low" water arsenic levels [35].

In the future, comparisons of study results will be facilitated if similar exposure metrics are used, and studies are more consistent in the way that exposure is handled analytically (*i.e.*, the boundaries used to define categories) and in the index of effect size used to convey the magnitude of the association between arsenic exposure and neurodevelopment.

4. Most Sensitive Endpoints

One of the more consistent findings is that increased arsenic exposure is associated with decrements in language-based skills, including Verbal IQ [29,32,39,40], verbal learning [32], digit span (memory for number strings) [36], story memory [32], and vocabulary [34]. On the other hand, inverse associations have also been reported for a variety of nonverbal endpoints, including Performance IQ and Processing Speed [33,31], individual subtests of the Wechsler scales that contribute to Performance IQ, such as Object Assembly (simple jigsaw puzzles) and Picture Completion (attention to visual detail) [34], visual-spatial abilities and sequencing [36], and motor skills such as body coordination and fine manual control [49].

Just as the diversity of exposure metrics used across studies impedes the identification of critical windows of vulnerability, it also makes it difficult to draw conclusions about the related issue of whether different neurodevelopmental domains are equally sensitive to arsenic. In principle, it is possible, even likely, that there are domain-specific windows of vulnerability, such that exposure during different developmental periods results in different phenotypes because of the different aspects of CNS development that occur at different ages [50]. Therefore, a comparison of the results of studies that relied on arsenic metrics with different exposure averaging times in an attempt to discern a "behavioral signature" for arsenic is not likely to be successful. As with lead, there might not be a single phenotype that occurs regardless of when arsenic exposure occurs [43].

The pattern of results in the prospective Bangladeshi study [37–39] are instructive with respect to future research needs in that inverse associations between prenatal arsenic exposure and neurodevelopment were observed when the children were tested at age 5 years but not at age 7 months or 8 months. This suggests the importance of continuing follow-up past infancy to ages when neurodevelopmental testing is considered to be more reliable and valid and when it is possible to assess domains, such as executive functions, memory, attention, that are difficult to evaluate in infants.

Although most studies have focused on cognitive endpoints, some have evaluated whether arsenic exposure is associated with behavioral dysfunctions in children. To date, the evidence is inconclusive. Using the Neonatal Behavioral Assessment Scale III, Parajuli *et al.* [42] found that the scores of Nepalese infants on one cluster of items (state regulation) were inversely related to arsenic, although scores on the other 6 clusters (habituation, orientation, motor system, state organization, autonomic stability, reflexes) were not. The prospective study of Bangladeshi children [37,38] found no significant associations between arsenic biomarkers and examiner ratings of infant behavior (activity, emotional tone, response to examiner, cooperation, vocalization) at 7 or 18 months of age. A cohort of

526 6–7 year olds residing near a smelter in Mexico [41] found what were described as "modest" associations between children's total urinary arsenic level and teachers' ratings of their behavior. Adjusting for child age, sex, maternal education, family socioeconomic status, ownership of home, crowding, blood lead, and hemoglobin, compared to children with urinary arsenic in the 1st quartile, children with levels in the 3rd and 4th quartiles had 2.4 (95% CI: 1.1–4.9) and 1.9 (95% CI: 0.9–4.3) times the likelihood of scoring in the range of clinical concern (≥65) on the ADHD Index of the Connors Behavior Rating Scale. Furthermore, greater exposure was associated with higher (worse) scores on teachers' ratings of Oppositional Behavior, Cognitive Problems, and ADHD Index. These associations were greatly attenuated when adjustments were made for children's scores on the Peabody Picture Vocabulary Test, a proxy measure of Verbal IQ, suggesting that the behavior problems of the children might have been a result of arsenic's adverse effects on cognition.

5. Potential Effect Modifiers

Two classes of factors that might influence arsenic neurotoxicity have been investigated: host characteristics and co-exposures to other neurotoxicants.

5.1. Host Characteristics

Several studies have examined whether the efficiency of arsenic methylation modifies the association between early life arsenic exposure and neurodevelopment, measuring the fraction of total urinary arsenic that is in the form of two metabolites, monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA). The assumption is that the lower the fraction of urinary arsenic that is in the form of DMA, or a higher fraction that is in the form of MMA, the less optimal is a child's capacity for methylation, and thus, the greater the risk of neurotoxicity. Although evidence supporting this hypothesis exists for cancer [51] and cardiovascular disease in adults [52], it has not consistently been shown to be true for neurotoxicity risk, although the way in which this issue has been addressed differs among studies. Hamadani *et al*. [39] reported that the percentage of urinary arsenic metabolites in the form of MMA did not alter the associations observed between arsenic exposure and neurodevelopment at age 5 years, and Wasserman *et al*. [31] found that the percentage of metabolites in the form of DMA was not associated with outcomes nor did it alter the associations between total urinary arsenic and the outcomes measured. In contrast, Roy *et al*. [41] reported that the concentration of DMA was associated with scores on the Cognitive Problems and ADHD Index subscales of a teacher-completed rating scale. For the ADHD Index, children in the third and fourth quartiles of DMA scored significantly worse than children in the first quartile. For the Cognitive Problems subscale, however, there was no clear dose-effect relationship, as only the scores of children in the second quartile of DMA were significantly worse than the scores of children in the first quartile. Concentration of MMA was not associated with any of the outcomes measured. Therefore, the importance of methylation efficiency in modifying the neurodevelopmental toxicity of arsenic remains unknown.

The evidence is similarly inconsistent with regard to a possible sex difference in vulnerability, a hypothesis stimulated, in part, by the evidence that arsenic interacts with many steroid hormones [51]. In the prospective Bangladeshi study [39], concurrent urinary arsenic was inversely associated with

Full-Scale and Verbal IQ in girls but not in boys. In contrast, Rosado *et al*. [36] found that urinary arsenic was inversely associated with more endpoints in boys (Visual-Spatial Abilities with Figure Design, Peabody Picture Vocabulary Test, Visual Search, Letter Sequencing) than in girls (Digit Span). With regard to behavior, Roy *et al*. [41] reported that greater urinary arsenic level was associated with worse scores on teacher-rated Cognitive Problems and ADHD Index in boys, but not in girls. Thus, the limited evidence of a sexual dimorphism in arsenic's developmental neurotoxicity is mixed.

5.2. Co-exposures

In recognition that arsenic-exposed children are often also exposed to other neurotoxicants, some studies have evaluated whether combined exposures are associated with greater toxicity than are exposures to arsenic alone. The two chemicals that have been examined in this way are lead and manganese. The rationale for examining these metals is provided by studies in rodents indicating that, compared to animals exposed to one metal at a time, combined exposure to arsenic, lead, and manganese resulted in lower brain levels of monoamines [53] and reduced dopamine response to stimulation [54], greater brain levels of delta-amino levulinic acid [55], greater white matter damage [56], reduction in glial fibrillary acid protein expression and increased apoptosis of astrocytes [57]. In three epidemiological studies, all conducted in Mexico, no evidence supporting a significant interaction between arsenic and lead was found [29,36,41]. In each of the studies, the mean blood lead level of the participants was around 10 µg/dL, which is well within the range that has been associated with neurotoxicity: 10.0 µg/dL [41]; 9.0 µg/dL in the high-arsenic group and 9.7 µg/dL in the referent group [29]; and 11.5 µg/dL [36]. This suggests the possibility that, in all three studies, children had lead exposure levels sufficient to produce neurotoxicity, perhaps limiting the ability to discern an interaction with arsenic exposure. The only evidence suggesting an interaction between arsenic and lead is provided by an ecologic study showing that the probability of intellectual disability in children is higher when the concentrations of both metals in the soil are higher [45].

Wright *et al*. [32] found significant interactions between hair arsenic and hair manganese levels for several outcomes, including Full-Scale IQ, Verbal IQ, verbal learning, and verbal memory. Significant main effects were found for both hair arsenic (mean 17.8, range 1.4–55.4 ng/g) and hair manganese (mean 471.5, range 89.1–2145.3 ng/g), but, for both metals, the main effect was heavily dependent on the poorer scores of children who had hair arsenic and manganese levels that placed them above the median value for each metal. The relative deficit of these children was about two-thirds of a standard deviation compared to the rest of the study sample. In contrast, Wasserman *et al*. [40] did not find a significant interaction between water arsenic and water manganese levels in a study conducted in Bangladesh, involving a cohort that likely experienced higher exposure to both arsenic and manganese than did the children studied by Wright *et al*. [32].

6. Modeling Issues

The decades-long history of epidemiological investigation of other metals such as lead and methylmercury provides several lessons that can guide the future conduct of research on arsenic neurotoxicity in children [58]. The first lesson is that neurodevelopment is a complex outcome that is

influenced by many factors other than metal exposures. In many early lead studies, conducted at a time when higher exposure was heavily confounded with socioeconomic deprivation, adjustment for sociodemographic covariates frequently reduced the effect size for lead by half or more. Whether arsenic exposure is similarly confounded or confounded to the same extent is unknown (and likely differs from study site to study site), but Wasserman *et al.* [40] noted in one of their Bangladeshi cohorts that the arsenic coefficients were, indeed, greatly diminished after adjustment for covariates that included maternal IQ, maternal age, duration of school attendance, head circumference, and serum ferritin. This suggests that careful attention must be given to identifying and measuring setting-relevant, and perhaps setting-specific, covariates and incorporating them into analyses in order to avoid drawing false inferences (positive or negative).

Two covariates that were found to be especially critical in lead studies were maternal IQ, which has been measured in few arsenic studies [40], and the extent to which a child's home environment promotes optimal cognitive development. The latter factor is typically assessed using the Home Observation for Measurement of the Environment (HOME), which is conducted by means of observation in the home and caregiver interview. Because this instrument was developed for U.S. populations, it must be adapted for use in developing countries in order to capture local caregiving practices. An adaptation of the HOME has been used in two Bangladeshi studies [40,39], and in both it was shown to be an important predictor of child neurodevelopment.

7. Conclusions

The pace of research on the developmental neurotoxicity of arsenic is increasing, with the current evidence providing few firm conclusions but ample reason to be concerned about the neurodevelopmental impact of this chemical. Given the large numbers of individuals worldwide who are at risk, it is possible that arsenic accounts for a significant fraction of the total neurodevelopmental morbidity in many regions [59].

A variety of methodological issues, primarily pertaining to exposure assessment, are obstacles to direct comparisons of the results of different studies and, thus, the drawing of inferences about many issues germane to the ability to conduct an arsenic risk assessment focused on neurodevelopment. These issues include, most notably, the existence of critical windows of vulnerability, characteristics of the dose-effect relationships (e.g., lowest-observed adverse effect level, functional form), and factors that identify subgroups at greatest risk. Progress in clarifying these issues will be facilitated if investigators adopt similar strategies for measuring children's internal and external exposure to arsenic during different developmental epochs, including gestation. Although the diverse cultural and linguistic settings in which studies are conducted makes the use of a standard battery of outcomes challenging, measurement of a core set of endpoints would make study findings more comparable, enabling more rapid progress in elucidating the key risk assessment issues noted above.

Acknowledgments

Conflicts of Interest

The author declares no conflict of interest.

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