

The Developmental Neurotoxicity of Arsenic: Cognitive and Behavioral Consequences of Early Life Exposure

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ABSTRACT

Background: More than 200 million people worldwide are chronically exposed to arsenic. Arsenic is a known human carcinogen, and its carcinogenic and systemic toxicity have been extensively studied. By contrast, the developmental neurotoxicity of arsenic has been less well described. The aim of this review was to provide a comprehensive review of the developmental neurotoxicity of arsenic.

Methods: We reviewed the published epidemiological and toxicological literature on the developmental neurotoxicity of arsenic.

Results: Arsenic is able to gain access to the developing brain and cause neurotoxic effects. Animal models link prenatal and early postnatal exposure to reduction in brain weight, reductions in numbers of glia and neurons, and alterations in neurotransmitter systems. Animal and in vitro studies both suggest that oxidative stress may be a mechanism of arsenic neurotoxicity. Fifteen epidemiological studies indicate that early life exposure is associated with deficits in intelligence and memory. These effects may occur at levels of exposure below current safety guidelines, and some neurocognitive consequences may become manifest only later in life. Sex, concomitant exposures, and timing of exposure appear to modify the developmental neurotoxicity of arsenic. Four epidemiological studies failed to show behavioral outcomes of arsenic exposure.

Conclusions: The published literature indicates that arsenic is a human developmental neurotoxicant. Ongoing and future prospective birth cohort studies will allow more precise definition of the developmental consequences of arsenic exposure in early life.

Key Words: arsenic, behavioral effects, children's environmental health, cognition, developmental neurotoxicity, developmental origins of adult disease

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INTRODUCTION

The World Health Organization (WHO) estimates that more than 200 million people worldwide are chronically exposed to arsenic at levels above proposed safety standards.¹ Contaminated ground water is the principal route of exposure, but airborne exposures make additional contributions, especially in the vicinity of mines, smelters, and industrial "hot spots."

Contaminated drinking water is the main route of human exposure to arsenic. Widespread contamination

of ground water by arsenic has been reported in Bangladesh, West Bengal, China, Taiwan, Thailand, Ghana, Argentina, Chile, Mexico, Hungary, Canada, the United Kingdom, and areas of the United States.² Its presence in the environment can be a result of natural sources, such as erosion or geological leaching, or anthropogenic sources, including mining, industrial wastes, and use of fertilizers containing arsenic.

Other less common sources of arsenic exposure include coal combustion and incineration of arsenic-preserved wood products. Consumption of tainted foods, ingestion of kitchen dust, inhalation of indoor air polluted by coal combustion, tobacco smoke, and hand-to-mouth soil ingestion have been reported as additional routes of arsenic exposure.^{2,3} Airborne exposures, especially in the vicinity of mines, smelters, and industrial "hot spots," make further contributions.

Arsenic has been shown to cross the placenta, and studies have also shown that in utero exposure may occur. One study used arsenic-labeled arsenate and arsenite in pregnant mice and by using autoradiography

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and gamma counting, observed that the arsenic passed through the placenta from the maternal to the fetal circulation.⁴ Other studies have also shown transplacental arsenic transfer in animal models.^{5,6} Similar findings have been described in humans. Strong positive correlations have been found between cord and maternal blood arsenic levels in arsenic-exposed pregnant women,⁷ and it has been demonstrated that levels of arsenic in cord and maternal blood were nearly identical among pregnant women living in an arsenic-contaminated area, suggesting virtually free passage of arsenic across the placenta from the mother to the fetus.⁸ Individual arsenic metabolites have been found to be strongly correlated in cord and maternal blood (inclusive of dimethylarsinate, monomethylarsonate, arsenite, and arsenate).⁷ These findings suggest the developing fetus is at risk for arsenic exposure via placental transfer.

Breast milk is another potential route of exposure for young children. However, animal studies have failed to show arsenic in breast milk. In a study of low-dose drinking water arsenic exposure, no arsenic was measured in the breast milk of exposed mice.⁹ It has been demonstrated that arsenic does not appear to pass into human breast milk, and thus was hypothesized that breast-feeding may therefore protect against arsenic exposure in Bangladeshi infants.¹⁰

Once arsenic gains access to the neonate, however, it may cross the blood–brain barrier (BBB) and directly affect the central nervous system (CNS). The BBB is a structure composed of tight junctions between capillary endothelial cells in the brain and epithelial cells in the choroid plexus specialized to prevent proteins and smaller molecules from mixing with the cerebrospinal fluid.¹¹ The neurotoxicity of a toxicant therefore depends in part on the permeability of the BBB to that toxin.¹² In the case of arsenic, animal studies have shown that brain arsenic levels have a dose–response relationship to levels of arsenic in drinking water, demonstrating that the BBB does not effectively block the passage of arsenic to the CNS.^{12–16} Additionally, it has been reported that arsenic may have a direct toxic impact on the BBB itself. A study on the effects of mixed metals on the BBB,¹² showed that various metals, arsenic among them, disrupted the integrity of the BBB likely via effects on astrocytes, leading to increased permeability of the BBB to toxicants such as arsenic.

Exposure Guidelines and Effects on Health

Major scientific reviews of arsenic toxicity by the National Academy of Sciences, the National Drinking Water Advisory Council, and the U.S. Environmental Protection Agency's (EPA) Science Advisory Board have confirmed that arsenic can have negative effects on human health at lower levels than previously suspected. These findings led the EPA to reduce the acceptable level of arsenic in drinking water from 50 to 10 µg/L. Health

Canada has proposed that the maximum acceptable concentration for arsenic in drinking water in Canada should be 5 µg/L, and as an interim measure has lowered its guidelines from 50 to 25 µg/L while technologies are developed to further reduce arsenic in drinking water.^{2,17} Similarly, the WHO has stated that the guideline for arsenic in drinking water would be 0.17 µg/L based on acceptable excess lifetime cancer risk of 10⁻⁵, but as this value is below the practical quantification limit, 10 µg/L was accepted as the provisional guideline value.¹⁸

The health effects of chronic, low-level exposure to arsenic include skin pigmentation, hypertension, cardiovascular disease, diabetes, anemia, reproductive effects, developmental effects, immunologic effects, and neurological disorders.^{2,18,19,20} Arsenic is also a carcinogen.²¹ The International Agency for Research on Cancer (IARC) considers arsenic a proven human carcinogen that definitely causes cancers of the lung, urinary bladder, and skin and additionally is linked to cancers of the kidney, liver, and prostate.²² Infants and children appear to be especially vulnerable to these effects, likely because of their greater consumption of food and drinking water on a body-weight basis than adults.²³

Although the systemic and carcinogenic toxicities of arsenic have been studied in detail, its neurocognitive consequences have not been extensively explored. The aim of this review was to evaluate and synthesize the findings of the available epidemiological, animal, and *in vitro* studies on the intellectual and behavioral effects of chronic arsenic exposure, especially of exposures in early life.

METHODS

A literature review was performed, using PubMed, library catalogues, and journals available at the Icahn School of Medicine at Mount Sinai Levy Library. These sources were searched using key words such as: arsenic or arsenic* and intellectual, cognitive, neuro*, neurotox*, intelligence, neurol*, IQ, behavior*, neurobehavior*. All types of relevant animal, *in vitro*, and human studies were considered including journal articles, reports, and book chapters, with an emphasis on more recent and more robust studies, and a focus on early life and prenatal arsenic exposure. Articles not written in English were excluded.

Studies Included

Ultimately, 47 papers were included in this review, made up of animal studies, *in vitro* studies using animal tissues, *in vitro* studies using human tissues, epidemiological studies, and meta-analyses. These 47 papers included only those used to address the question of the neurotoxic effects of arsenic, and did not include those cited as part of the introduction or as background citations in the body of the manuscript not directly related to the focus of this review (see [Table 1](#) for studies included by type).

Table 1. Studies Included by Type

Paper Type	Animal Study	In vitro Study, Animal Tissues	In vitro Study, Human Tissues	Epidemiological Studies	Meta-analysis of Cross-Sectional Human Studies	Total
Number of papers included	14*	4*,†	2*,†	28	2	47

*Chattopadhyay et al.³⁵ is included in the categories of animal study, in vitro study using animal tissues, and in vitro study using human tissues, as it studied in vivo and tissue cultures of rat brains and human fetal explant brain tissue.

†Chattopadhyay et al.³⁶ studied both in vitro rat and human tissue, and thus is included in the categories of in vitro study of both human and animal tissues.

ANIMAL AND IN VITRO MODELS: ELUCIDATING MECHANISMS OF ARSENIC TOXICITY

Although epidemiological studies are critical to documenting the neurobehavioral consequences of arsenic exposure in humans, they allow critical scrutiny of the potential mechanisms of these effects.

Neurobehavioral Effects in Animal Models

Arsenic has been documented in behavioral and cognitive testing to have functional effects on the CNS in experimental animals. In a study of weanling rats, Spatial learning ability was tested after arsenic exposure using a battery of tests, including hidden platform acquisition tests and visible platform trials.¹³ The study results concluded that arsenic exposure was related to impairment in spatial memory as determined by poorer performance on hidden platform acquisition tests. A similar study of arsenic exposure from the prenatal period until around 4 months of age showed that exposed rats had increased errors in a delayed alternation task that required the use of sensory information,¹⁴ and another study found that arsenic-exposed offspring had deficits in spatial working memory and reactivity to novelty.²⁴

Additional studies have explored arsenic's effects on learning and conditioning in developing animals. In one study, both adult and infant rats were subjected to a variety of operant conditioning tests. The study demonstrated that developing rats exposed to arsenic demonstrated learning deficits, and that they required more sessions to acquire a learned task compared with unexposed controls.²⁵ Acquisition of new skills in adult animals was not affected by arsenic exposure. This differential effect on learning in young animals compared with adult animals implies the existence of windows of susceptibility, with the developing brain likely more susceptible to arsenic's neurotoxic effects. Furthermore, even after a rehabilitation period of 100 days without exposure, developing rats continued to show deficits in learning, a finding that suggests a level of irreversibility. In another test of extinction of learned behavior, both adult and developing arsenic-exposed animals took

longer to extinguish a conditioned behavior than controls, highlighting that although the developing brain may be more susceptible to arsenic's effects, the adult brain is not immune. The study concluded that arsenic has effects on learning, especially in the developing brain, and these effects have limited reversibility.²⁵

Effects on Body and Brain Weight

Dose-dependent reductions in body and brain weight are outcomes of arsenic exposure that have been shown repeatedly in studies of young, arsenic-exposed animals.^{9,13-16,25} The mechanisms responsible for these reductions in body and brain weight are not fully understood. It was previously found that the decrement in weight gain in arsenic-exposed developing rats was not related to decreased food intake.¹³ Arsenic exposure has been shown to be accompanied by decreased body and brain weights in adult rats as well, suggesting that these effects are not restricted to early development.²⁵ Curiously, although one study demonstrated a growth deficiency in arsenic-exposed mice, it was found that cross-fostering exposed pups to a mother without arsenic exposure could reverse this deficit.⁹ This finding led to the hypothesis that the growth deficit may be due to nutritional deficiency in the milk of an arsenic-exposed mother.⁹ To support this hypothesis, an analysis of breast milk from arsenic-exposed mice showed decreased nutrient content, specifically triglycerides, even at low doses of arsenic (10µg/L).⁹ The significance of decreased body weight and head circumference lies with the finding that reduced head circumference at birth is a reflection of diminished brain growth in utero and has been associated with lower child intelligence.²⁶

Effects on Neurotransmitter and Signaling Systems

Arsenic appears to have toxic effects on neurotransmitters involved in cell-to-cell signaling within the brain. A study of rats demonstrated that arsenic induced regional increases in levels of dopamine, serotonin, and their metabolites and also induced a decrease in norepinephrine levels in discrete brain regions.²⁷ Interestingly, the study found that the increases in dopamine and serotonin were paralleled by increases in their metabolites,

suggesting both synthesis and metabolism of the neurotransmitters were augmented by arsenic exposure.²⁷ In another study,²⁵ consumption of inorganic arsenic by rat pups from postnatal day 2 to 60 days of age led to decreases in acetylcholinesterase (AChE) activity, an enzyme critical for the metabolism of acetylcholine, another important neurotransmitter. AChE decreases were seen even after allowing for a recovery period without ongoing exposure to arsenic, suggesting an element of irreversibility.²⁵ Another study found arsenic to cause regional alterations in the levels of glutamate, γ -aminobutyric acid, and other brain biogenic amines.²⁸

The full biological significance of these changes in neurotransmitter levels induced by arsenic is not yet clear, but a previous study¹³ suggested that these alterations may precede the appearance of cognitive effects. This study conducted learning and spatial memory tests on weanling rats exposed to arsenic, and also studied the glutamate receptor *N*-methyl-D-aspartate receptor (NMDAR) as well as ultrastructural changes in the hippocampus. NMDAR was examined because it plays a role in synaptic plasticity and learning, and both NMDAR and the hippocampus are thought to be involved in memory. The investigators found that arsenic exposure was associated with dose-dependent decreases in the expression of certain NMDAR subunits as well as with hippocampal ultrastructural changes. Notably, although only the highest arsenic-exposure group showed a deficit in the spatial memory test, the ultrastructural and molecular changes noted were dose-dependent and were found even at low levels of arsenic exposure. These intriguing findings suggest that these ultrastructural and protein-expression changes were more sensitive markers for arsenic exposure than the neurobehavioral changes,¹³ perhaps preceding the observable neurobehavioral effects.

In addition to the neurotransmitter system, the hormonal system may be affected as well. Perinatal-exposed mice have been found to have higher basal levels of the steroid hormone corticosterone compared with controls.²⁹ It has also been shown that perinatal exposure led to reductions in glucocorticoid receptor abundance in the hippocampus.²⁴ As a study of glucocorticoid receptor knockout mice reported impaired spatial memory in the animals,³⁰ this reduction in glucocorticoid receptor abundance may have intellectual implications.

Cellular Impacts: Glial and Neuronal Effects

Arsenic has been shown in animal models to cause cellular changes in the brain. In a study that examined arsenic and other neurotoxic heavy metals, it was demonstrated that arsenic increased apoptosis in astrocytes, reduced astrocytic processes, and reduced astrocytic expression of glial fibrillary acidic protein, an intermediate filament protein thought to be important to

maintenance of astrocyte shape and strength.¹² As astrocytes are vital to the integrity of the BBB, provide biochemical support to the nervous tissue, and are thought to be important in long-term potentiation and memory formation, these effects may have implications for the neurobehavioral outcomes of arsenic.

Neurons also are susceptible to arsenic toxicity, and one study showed that sodium arsenite reduced cerebellar neuron viability and induced DNA degradation and nuclear fragmentation in cultures of rat cerebellar neurons.³¹ It has been shown that, in cultured mouse neuronal cells, sodium arsenite led to neuronal apoptosis, necrosis, and inhibited neurite growth in a dose-dependent manner.³² Arsenic exposure in rats resulted in individual shrunken cells with condensed cytoplasm and nucleus in the hippocampus; mitochondrial changes were noted, edema was seen around capillaries, and there were decreased synaptic vesicles in the synapses.¹³ All of these effects were dose-dependent.¹³

Oxidative Stress: A Possible Mechanism of Arsenic Neurotoxicity

Oxidative stress has been implicated as a mechanism responsible for arsenic's carcinogenic potential in many organ systems, and may likewise play a role in the metalloid's neurotoxicity. It has been shown that arsenic, along with several other heavy metals, generates reactive oxygen species (ROS) in developing rat brains.¹² In another study,¹⁶ sodium arsenite in drinking water was found to lead to the generation of ROS and subsequent lipid peroxidation in the brains of developing rat pups.

In addition to demonstrating ROS generation, the pups' levels of the antioxidant glutathione (GSH) as well as the activity of the antioxidant enzyme glutathione peroxidase (GPx) were reduced following arsenic exposure.¹⁶ GSH and GPx play essential roles in protecting an organism from oxidative damage; a reduction in these reduces the capacity of an organism to defend itself from the damage caused by ROS. In another study focusing on oxidative stress, Rats administered arsenic in drinking water, even at the "permissible limit" (50 $\mu\text{g/L}$, the national standard in Bangladesh), displayed increased lipid peroxidation, decreased GSH levels, and reduced superoxide dismutase and glutathione reductase activities in the brain, indicating free radical-mediated cellular degeneration.³³ Using human fetal brain explants, one study^{34,35} showed similar effects in human cells in vitro: Arsenic exposure induced disturbances in the GSH-cycle enzymes and in neuronal development, and caused generation of reactive oxygen nitric oxide species and apoptosis of fetal neuronal cells.

Concurrent Heavy Metal Exposures

Concurrent heavy metal exposures may exacerbate the neurocognitive effects of arsenic. A study of rats exposed to heavy metals demonstrated that concurrent exposure to lead and arsenic led to alterations in norepinephrine

in the hippocampus and serotonin in the midbrain and cortex, alterations that were not seen in groups exposed to either element alone.²⁷ In a functional study of rats exposed to metals during brain development, more than additive effects on learning-memory performance, integrity of the BBB, apoptosis, morphological changes, and ROS generation were seen in animals simultaneously exposed to mixtures of cadmium, lead, and arsenic than in animals exposed to individual elements.¹² These findings set up a precedent for observations in epidemiological studies of multimetal exposure in humans as discussed later.

EPIDEMIOLOGICAL STUDIES

Epidemiological studies have also begun to suggest that low-level and chronic exposure to arsenic is associated with serious effects on intellectual function across a broad age range. Twenty epidemiological studies assessed for neurocognitive or behavioral outcomes associated with arsenic; 17 of them focused on neurocognitive outcomes and of these, 15 showed neurocognitive or intellectual deficits associated with arsenic exposure, while 2 failed to show effect. Three epidemiological studies focused wholly on behavioral outcomes, and 1, included in the 17 above, assessed for behavioral alongside intellectual outcomes, and none of these showed significant behavioral effects of arsenic exposure (Table 2).

In children, adverse neurobehavioral outcomes have been associated with acute and chronic arsenic exposure. A meta-analysis in arsenic-exposed children indicated intelligence deficits; considering 4 cross-sectional studies in China on arsenic exposure and IQ effects, this analysis found that the overall mean IQ score of children who lived in arsenic-exposed areas was more than 6 points lower than the mean score in unexposed children.³⁶ Indeed, a growing number of studies are confirming intellectual deficits associated with arsenic exposure in children as young as 5 years of age³⁷⁻⁴⁶ (Table 2).

A cross-sectional study of an older age group likewise described neurobehavioral effects related to chronic arsenic exposure in adolescents.⁴⁷ The study found that adolescents exposed to arsenic-contaminated well water in early life performed more poorly in 3 of 4 neurobehavioral subtests compared with unexposed controls, suggesting that childhood exposure to arsenic might affect neurobehavioral development in later life.

A study of a geriatric population showed that long-term low-level arsenic exposure (average 6.33 µg/L, a level below the current safety guideline of 10 µg/L) in adults as estimated by geographic information system (GIS)-based models was significantly associated with poorer global cognition, diminished visuospatial skills, reduced language, slower processing speed, impaired

executive functioning, and diminished short-term memory.⁴⁸ Although the exposure modeling in this study may have introduced error, as the GIS-estimated versus measured arsenic levels in wells were not always in accord,⁴⁸ it suggests that the intellectual effect of arsenic spans age ranges from young to old.

Not all studies clearly demonstrated a relationship between arsenic exposure and neurodevelopmental toxicity. A study of prenatal arsenic-exposed children in Bangladesh, failed to demonstrate any deficit in problem-solving abilities or motor development in 7-month-old children.⁴⁹ A cross-sectional assessment of a longitudinal cohort study in rural Bangladesh found no significant effect of arsenic exposure as shown by maternal and child urinary arsenic concentrations to child development measures at 18 months of age.⁵⁰ However, in a subsequent study, prenatal maternal arsenic as well as ongoing arsenic exposure was found to have a negative effect on intellectual functioning in the same cohort of children in Bangladesh at 5 years, suggesting that early arsenic may have an effect on the brain that is only later revealed through intellectual testing. This effect may be sustained or aggravated by continuing exposure.⁵¹

Neurocognitive and Behavioral Elements Affected

Although epidemiological studies commonly find that arsenic exposure early in life leads to deficits in full-scale IQ, verbal IQ, and memory, the particular intellectual areas found to be most affected are not entirely consistent across studies (Table 2). Higher tertiles of water arsenic exposure resulted in 6-year-old children performing more poorly in tests of full-scale and processing speed, whereas verbal scores did not appear to be affected.⁴⁴ By contrast, in a study of 11- to 13-year-old youth in Oklahoma, the functional domain most consistently affected was verbal skills, including memory.⁴⁶ A study of 6- to 8-year-old Mexican schoolchildren similarly suggested that arsenic toxicity principally affects memory, problem-solving, and attention span.⁴⁰ A longitudinal cohort study of children in Bangladesh suggested decrements in verbal IQ and full-scale IQ were associated with arsenic exposure.⁵¹ Urinary arsenic has been inversely associated with full and verbal IQ in 6- to 8-year-old Mexican children.³⁷ Finally, arsenic in drinking water has been associated with decreased full IQ scores in children 6- to 10 years of age.³⁹ The inconsistencies across studies in the areas affected by arsenic may reflect differences in study design, sample characteristics, levels of exposure, the intellectual tests used, the markers of exposure examined, and the ages of study participants (Table 2).

Despite some inconsistencies in outcome, most studies focusing on neurocognitive outcomes did find deficits associated with arsenic exposure. In a meta-analysis including 15 studies assessing arsenic exposure and neurodevelopment, 13 articles showed a significant

Table 2. Epidemiological Studies of Effects of Arsenic Exposure on Neurocognitive or Behavioral Outcomes (N = 20)

Location	Study Population Size	Age of Population (y)	Study Design	Exposure Metrics	Exposure Levels (mean)	Psychological Tests/Outcome Used*	Effects Observed	Author and Year of Publication
Mexico	41	6-9	Cross-sectional	Creatinine-adjusted Urinary As	62.9 µg/g creatinine	<i>Intellectual:</i> WISC-RM	↓ Verbal IQ, long-term memory, linguistic abstraction	Calderón et al. 2001 ³⁷
India	351	5-15	Cross-sectional	Urinary As	78 µg/L	<i>Intellectual:</i> WISC, RCPMT, Total Sentence Recall Test	↓ Vocabulary, object assembly, picture completion	Ehrenstein et al. 2007 ³⁸
Bangladesh	2112	1.5	Longitudinal cohort	Maternal urinary As averaged over early and late gestation; Child urinary As	96 µg/L maternal UAs; 35 µg/L child UAs	<i>Intellectual:</i> BSID-II, WBRS and maternal report of language	None significant	Hamadani et al. 2010 ⁵⁰
Bangladesh	2260	5	Longitudinal cohort	Maternal urinary As in late gestation; Child urinary As	84 µg/L maternal UAs; 51 µg/L child UAs	<i>Intellectual:</i> WPPSI	Maternal UAs ↓ performance IQ, child UAs ↓ verbal IQ, full-scale IQ in girls only	Hamadani et al. 2011 ⁵¹
Bangladesh	201	8-11	Cross-sectional	Water, blood, urinary As	Water As: 43.7 µg/L; blood As 5.1 µg/L; UAs 75.2 µg/L	<i>Behavioral:</i> CBCL	None significant	Khan et al. 2011 ⁵⁴
United States	12052	Mean follow-up to 6.7 years	Retrospective cohort	Soil As estimates	3.3 mg/kg dw	<i>Intellectual:</i> Unknown Cause Intellectual Disability Healthcare Diagnosis	First trimester exposure to elevated soil As increased odds of intellectual disability diagnosis	McDermott et al. 2012 ⁵⁹
United States	434	40-85	Longitudinal cohort	Current estimated ground water As	6.33 µg/L	<i>Intellectual:</i> MMSE, EXIT25, RBANS, TMTA, COWAT	↓ Language, visuospatial skills, executive functioning, global cognition, immediate memory	O'Bryant et al. 2011 ⁴⁸
Mexico	132	6-10	Cross-sectional	Creatinine-adjusted urinary As, drinking water As	UAs 12.6, 116, 52.5 µg/g creatinine [†] ; Drinking water As 5.8, 169, 194 µg/L [†]	<i>Intellectual:</i> WISC-RM	UAs ↓ full-scale IQ, drinking water As ↓ performance IQ, verbal IQ, full-scale IQ	Rocha-Amador et al. 2007 ³⁹

Mexico	602	6-8	Cross-sectional	Urinary As	58.1 µg/L	<i>Intellectual:</i> WISC-RM, CAT, NLST, VSAFD, PPVT, MAT	↓ Memory, problem solving, attention	Rosado et al. 2007 ⁴⁰
Mexico	526	6-7	Cross-sectional	Urine As	52.5 µg/L	<i>Behavioral:</i> CPRS-R, CTRS-R	None significant	Roy et al. 2011 ⁵⁵
Thailand	529	6-9	Cross-sectional	Hair As	3.52 µg/g	<i>Intellectual:</i> WISC-III	↓ full-scale IQ	Siripitaya-kunkit et al. 1999 ⁴¹
Taiwan	109	12-14	Cross-sectional	Estimated cumulated As exposure in water	520629, 13782.2, 0 mg/L [‡]	<i>Intellectual:</i> CPT, SD, PM, SA	↓ continuous performance, pattern memory, switching attention	Tsai et al. 2003 ⁴⁷
Bangladesh	1799	7 months	Longitudinal cohort	Maternal urinary As (at 8 and 30 wk pregnancy)	Maternal UAs 81 µg/L at 8 wk, 84 µg/L at 30 wk (medians)	<i>Intellectual:</i> PSTs, motor scale of BSID-II. <i>Behavioral:</i> behavior ratings	None significant	Tofail et al. 2009 ⁴⁹
China	720	8-12	Cross-sectional	Well water As	190, 142, 2 µg/L [§]	<i>Intellectual:</i> CRT-RC ₂	↓ general IQ	Wang et al. 2007 ⁴²
Bangladesh	201	10	Cross-sectional	Well water As, urinary As	Water As 117.8 µg/L, UAs 116.6 µg/L	<i>Intellectual:</i> WISC-III	Water As ↓ performance IQ, verbal IQ, full-scale IQ	Wasserman et al. 2004 ⁴³
Bangladesh	301	6	Cross-sectional	Well water As, urinary As	120.1 µg/L, UAs 110.7 µg/L	<i>Intellectual:</i> WISC-III	↓ performance IQ, full-scale IQ, processing speed IQ	Wasserman et al. 2007 ⁴⁴
Bangladesh	299	8-11	Cross-sectional	Blood As, Creatinine-adjusted urinary As	Blood As 4.81 µg/L, creatinine-adjusted UAs 246.54 mg/g creatinine	<i>Intellectual:</i> WISC-IV	Blood As ↓ all subset scores particularly full-scale IQ, working memory, verbal comprehension; UAs ↓ all subset scores particularly verbal comprehension	Wasserman et al. 2011 ⁴⁵

(continued)

Table 2 (continued). Epidemiological Studies of Effects of Arsenic Exposure on Neurocognitive or Behavioral Outcomes (N = 20)

Location	Study Population Size	Age of Population (y)	Study Design	Exposure Metrics	Exposure Levels (mean)	Psychological Tests/Outcome Used*	Effects Observed	Author and Year of Publication
United States	31	11-13	Cross-sectional	Hair As	17.8 µg/kg	<i>Intellectual:</i> WASI, WRAVMA, CCT, CVLT-c, WRAML. <i>Behavioral:</i> CDI, BASC, CADS-IV, BRIEF.	↓ full-scale IQ, verbal IQ, memory	Wright et al. 2006 ⁴⁶
United Arab Emirates	92	5-15	Matched case –control	Blood As	Not reported	<i>Behavioral:</i> CTRS-CPRS, CBCL, DSM-IV	None significant	Yousef et al. 2011 ⁵³

As, arsenic; BASC, Behavior Assessment System for Children; BRIEF, Behavior Rating Inventory of Executive Functions; BSID-II, Bayley Scales of Infant Development-II; CADS-IV, Conners' ADHD DMS-IV Scales; CAT, Cognitive Abilities Test; CBCL, Child Behavior Checklist; CCT, Children's Category Test-Level II; CDI, Children's Depression Inventory; CELF-3, Clinical Evaluation of Language Fundamentals-Third Edition; COWAT, Controlled Oral Word Association Test; CPRS, Conners' Parent Rating Scale; CPRS-R, Conners' Parent Rating Scale—Revised; CPT, Continuous Performance Test; CRT-RC₂, Combined Raven's Test – The Rural in China Method; CTRS, Conners' Teachers Rating Scale; CTRS-R, Conners' Teachers Rating Scale—Revised; CVLT-c, California Verbal Learning Test-Children; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; EXIT25, Exit Interview; MAT, Math Achievement Test; MMSE, Mini Mental Status Examination; NLST, Number and Letter Sequencing Test; PM, Pattern Memory; PPVT, Peabody Picture Vocabulary Test; PSTs, Problem Solving Tests; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; RCPMT, Raven Colored Progressive Matrices Test; SA, Switching Attention; SD, Symbol Digit; TMTA/TMTB, Trails Making Test; UAs, urinary arsenic; VSAFD, Visual-Spatial Abilities with Figure Design; WASI, Wechsler Abbreviated Intelligence Scale; WBRs Wolke's Behavior Rating Scale; WISC, Wechsler Intelligence Scale for Children; WISC-III, Wechsler Intelligence Scale for Children-Third Edition; WISC-IV, Wechsler Intelligence Scale for Children-Fourth Edition; WISC-RM, Wechsler Intelligence Scale for Children-Revised Mexican Edition; WPPSI, Wechsler Pre-school and Primary Scale of Intelligence; WRAML, Wide Range Assessment of Memory and Learning; WRAVMA, Wide Range Assessment of Visual Motor Ability.

*Psychological tests/outcomes used are classified as either intellectual or behavioral (in italics).

[†]Three means given for 3 distinct communities included in this study.

[‡]Calculated as well water (mg/L) × drink year (y) × 365 (d) × volume (cm³) × 10⁻³; 3 values given for high exposure, low exposure, and control groups.

[§]Three values given for high exposure, low exposure, and control groups.

negative effect on neurodevelopment in children between the ages of 5 and 15 years. In separate meta-analyses on the results of articles that assessed arsenic exposure in urine ($n = 6$) and those that studied drinking water ($n = 4$), it was found that the combined magnitude of effect suggests that a 50% increase in arsenic levels in urine causes a decrease of 0.39 points in full-scale IQ ($P = .09$), whereas a 50% increase in water arsenic would cause a significant decrease ($P = .052$) of 0.65 points in the full-scale IQ.⁵²

Several studies also have examined the behavioral effects of arsenic exposure. A study of elementary school children in United Arab Emirates, found no significant relationship between levels of arsenic exposure and the odds ratio of having attention-deficit hyperactivity disorder (ADHD).⁵³ Likewise, no significant associations between markers of arsenic exposure and classroom behavior outcomes, including internalizing and externalizing outcomes, were found in a cross-sectional component of an ongoing study of children in Araihaar.⁵⁴ In a cross-sectional study of 11- to 14-year-old children in Oklahoma, a state known to have high background levels of environmental arsenic, parents' and teachers' rating of children's behavior using validated scoring systems were not consistently associated with hair arsenic levels.⁴⁶ Similarly, the same children's hair metal arsenic levels and their self-reports on behavioral tests were not significantly associated.⁴⁶ A study in Bangladesh failed to detect any deficit in behavior tests in 7-month-old infants with prenatal arsenic exposure.⁴⁹ In Torreón, Mexico, a study of 526 children aged 6 to 7 years found that, although arsenic markers were not associated with parental rating of behavior, higher urinary arsenic was associated with higher scores of oppositional, cognitive problems, and ADHD subscales.⁵⁵ Further analysis showed that, after adjustment for the Peabody Picture Vocabulary Test scores, the relationship between urinary arsenic and behavior became statistically nonsignificant, suggesting the harmful effects of arsenic on behavioral outcomes may be largely secondary to arsenic-induced cognitive effects.⁵⁵ The behavioral literature is relatively limited, but these studies taken together suggest the need for further elucidation of behavioral effects of arsenic exposure.

Effects on Body Weight and Head Circumference

In an ecological study conducted in Taiwan, newborns born in a historically arsenic-exposed area had an average birth weight 30 g lower than those born in a non-arsenic-exposed area; however, potential confounders such as maternal nutrition and tobacco use, as well as individual water arsenic levels and arsenic biomarkers, were not considered.⁵⁶ In a prospective cohort study of pregnant women in Matlab, Bangladesh, anthropometric measurements of newborns were taken after maternal

urinary arsenic was measured at 8 and 30 weeks gestation. Although analysis over the full range of average arsenic exposure found no dose-effect of arsenic on anthropometric measures, when only low levels of average arsenic exposure ($<100 \mu\text{g/L}$ urinary arsenic) were included, significant dose-dependent reductions in body weight, head circumference, and chest circumference at birth associated with prenatal arsenic exposure were shown.⁵⁷ A study aimed at elucidating the effects of arsenic in early and later gestation similarly found that, in a population-based mother-child cohort in rural Bangladesh, maternal arsenic exposure caused decreases in fetal size, with main decreases occurring below $100 \mu\text{g/L}$ urinary arsenic and primarily affecting head size and femur length.⁵⁸ Reduced head circumference at birth is a reflection of diminished brain growth in utero and has been associated with lower child intelligence.²⁶

Timing and Duration of Exposure

Timing of exposure to arsenic seems to affect outcome. A retrospective cohort study of pregnant women, in which soil arsenic concentration during each month of pregnancy and early childhood was used as a measure of environmental exposure found that only in the first trimester of pregnancy was elevated soil arsenic concentration associated with increased odds of diagnosed intellectual disability (ID), with no increased odds of ID later in pregnancy or in early childhood up to 2 years of age.⁵⁹ However, the study's limited population, reliance on health care diagnoses of ID, and a modeling estimation approach for soil arsenic concentration may have introduced error.⁵⁹

In a study that directly quantified water arsenic exposure, above-median early prenatal maternal arsenic exposure in drinking water was found to be associated with a decreased verbal IQ in children, and late gestational maternal arsenic exposure was associated with a decreased performance IQ in children at 5 years of age.⁵¹ However, despite these associations between prenatal arsenic exposure and decreased intellectual functioning, the strongest association in IQ deficits was with concurrent arsenic exposure at time of assessment, suggesting that ongoing exposure is an important contributor to the neurological toxicity of arsenic.⁵¹ Recent arsenic exposure, rather than prenatal or early life exposure, has been demonstrated to be the most important determinant of decrements in intellectual testing associated with arsenic exposure.³⁸

Duration of exposure also may increase the magnitude of the neurotoxicity of arsenic. In two separate studies of children living in the same arsenic-contaminated area of Bangladesh, a similar pattern of decrements in IQ measures were found in 6-⁴⁴ and 10-year-old children,⁴³ with stronger associations between water arsenic levels and IQ outcomes in the older group. Similarly, in a cohort of children living in Bangladesh, intellectual effects associated with prenatal and ongoing

arsenic exposure at 5 years of age were observed⁵¹; whereas previous findings showed no decrements in child developmental measures at 18 months in the same cohort.⁵⁰ These findings may suggest that ongoing exposure to arsenic results in cumulative effects, or that there may be a latency period between arsenic exposure in early life and the time when the full neurological effects of this exposure become manifest. However, other factors, such as mitigation activities that occurred in the interim between the two studies in each case that potentially resulted in misclassification of exposure, and the decreased reliability of intellectual testing in younger children, must be considered as possible explanations for the apparent weaker effect in the younger cohorts.⁴⁴

Modulators of Arsenic Toxicity

It is increasingly recognized that a multitude of factors can influence the magnitude of the neurodevelopmental toxicity of arsenic. Sex appears to be one of these effect modifiers. Several studies have reported that urinary arsenic levels were higher in boys than girls despite comparable exposures.^{40,46} In a study on the association between arsenic exposure and cord blood DNA methylation, exposure in male newborns was positively associated with global DNA methylation, whereas in females the inverse relationship was found, although in the female group this relationship did not reach statistical significance.⁶⁰ results of a maternal–child cohort study in rural Bangladesh, found that maternal arsenic seemed to affect mainly male fetuses in anthropometric measures, including decreases in head size and femur length.⁵⁸ These differences may be due to sex-related metabolic differences, a hypothesis supported in a study showing that urinary arsenic metabolite profiles were different between sexes.⁶¹

Intellectual outcomes similarly differ by sex. A longitudinal cohort study in Matlab, Bangladesh found that arsenic exposure had much stronger adverse effects on intellectual functioning in girls than in boys.⁵¹ An animal study in mice supports this differential effect by sex, with female mice pups appearing to be more vulnerable than males to the developmental effects of arsenic exposure.⁹ As arsenic is a well-documented endocrine disruptor,⁶²⁻⁶⁴ the difference in effect on the 2 sexes during development may be another example of endocrine effects.⁹

Concurrent exposure to other heavy metals is a second factor that has been shown to influence the magnitude of the neurodevelopmental toxicity of arsenic in animal models as previously discussed, and synergistic detrimental effects have been noted with such exposures, yet very little research has been done in human populations. A cross-sectional pilot study of 11- to 14 year olds in Oklahoma aimed to explore the effects on intellectual scores of multimetal exposure. This study found that arsenic and manganese had more than additive effects on intellectual functioning, as children with hair levels of both manganese and arsenic above the median

values in the same showed the greatest deficits in functional domain testing.⁴⁶ As discussed earlier, animal studies corroborate these findings.

Limitations of the Discussed Epidemiological Studies

Although substantial progress has been made in documenting the neurobehavioral effects of prenatal and early life exposures to arsenic and the mechanisms underlying these toxic effects, gaps in understanding remain. These gaps are largely due to difficulties in exposure assessment and in evaluating the intellectual effects of arsenic in young children. Most epidemiological studies to date have been performed using a cross-sectional design due to the difficulty of following a cohort of children prospectively from the prenatal period. However, the cross-sectional design has inherent limitations. A major limitation is the necessity in cross-sectional studies of using estimation techniques to approximate cumulative exposure to arsenic, an approach to exposure reconstruction that introduces error into quantifying chronic exposure and tends to bias results toward the null.^{38,41,48} Another limitation in some of these studies is that education and remediation initiatives may have led children who were previously classified as “high exposure” to have switched over to a low-arsenic water source, thus contributing to misclassification of exposure.⁴⁴ Finally, the cross-sectional design cannot prove causation but only demonstrate association, as it cannot be shown definitively that the exposure preceded and produced the effect.

CONCLUSIONS

Although scientific understanding of the developmental neurotoxicity of arsenic is still evolving, epidemiological and toxicological studies show clearly that arsenic is a developmental neurotoxicant that affects intellectual function. These studies demonstrate additionally that exposures even below current safety guidelines are associated with decrements in intellectual function, often particularly involving full-scale IQ and memory.

The neurotoxic effects of arsenic appear to be most severe in the developing brain.^{25,47,51,59} Their detection may be difficult because some of the consequences of early exposure may become manifest only later in life when neuronal attrition associated with aging may unmask deficits caused by early exposures.⁶⁵

Myriad factors may modulate the developmental neurotoxicity of arsenic, including sex, concomitant heavy metal exposures, and timing of exposure. Animal and in vitro studies broadly corroborate human data on the developmental neurotoxicity of arsenic and have linked prenatal and early postnatal exposure to reduction in brain weight, reductions in number of glia and neurons, and alterations in neurotransmitter systems.

Animal and in vitro studies suggest that oxidative stress may be a mechanism of arsenic neurotoxicity.

Both current and future longitudinal prospective studies will help to further elucidate the developmental neurotoxicity of arsenic and to quantify risk, especially at lower levels of exposure. One such study is already underway: HEALS (Health Effects of Arsenic Longitudinal Study) has enrolled more than 20,000 men and women in Arai-hazar, Bangladesh to prospectively investigate the health effects of arsenic at low to moderate levels. HEALS has already increased the understanding of the health effects of arsenic, biomarkers of exposure, and the efficacy of mitigation programs.^{66,67} Findings from such studies will clarify the effects of arsenic exposure in children and their underlying mechanisms, allowing a refined definition of safe levels of exposure, a better understanding of pathogenesis and prevention, and more effective protection of the most vulnerable populations from the cognitive consequences of this pervasive poison.

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