Neurotoxicology and Teratology 72 (2019) 49–57

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Developmental neurotoxicity of inorganic arsenic exposure in Sprague-Dawley rats

Christopher L. Moore[a,1], Timothy J. Flanigan[a,1], Charles D. Law[a], Lucie Loukotková[b], Kellie A. Woodling[b], Gonçalo Gamboa da Costa[b], Suzanne C. Fitzpatrick[c], Sherry A. Ferguson[a,*]

[a] Division of Neurotoxicology, National Center for Toxicological Research (NCTR), FDA, Jefferson, AR 72079, United States of America
[b] Division of Biochemical Toxicology, NCTR, FDA, Jefferson, AR 72079, United States of America
[c] Office of the Center Director, Center for Food Safety & Applied Nutrition, FDA, College Park, MD 20740, United States of America

ARTICLE INFO

Keywords:
Inorganic arsenic
Arsenate
Neurobehavior
Developmental toxicity
Drinking water
Rat

ABSTRACT

High levels of inorganic arsenic (iAs) exposure are associated with severe health effects. Less clear are effects of lower exposure levels on neurodevelopment. Relative to maternal intake, there is limited lactational transfer of arsenic in humans or rodents, yet there are few rodent studies which directly exposed preweaning animals. To more clearly determine iAs developmental neurotoxicity, 28 pregnant Sprague-Dawley rats were exposed to arsenate (AsV) via drinking water (0, 23.6, 47.7, 71.0 ppm) (n = 5–7/group) from gestational day (GD) 6 through GD 22 with targeted doses of 0, 2.33, 4.67, 7.00 mg/kg/day, respectively. Offspring were dosed by gavage daily with the same mg/kg AsV dose as intended for their dam from postnatal day (PND) 1 to 21. Gestational water intake was reduced at all AsV doses, but returned to control levels on lactational day (LD) 1 when control water was returned. Gestational body weight was reduced only at the highest dose on GD 22 and lactational body weight was unaffected. Food intake was unaffected. iAs exposure did not alter offspring body weight (PNDs 1–21) or age at fur development and bilateral ear opening. Incisor eruption, however, was significantly delayed in offspring of the 4.67 and 7.00 mg/kg groups. Further, all iAs groups were significantly delayed in bilateral eye opening. Righting reflex (PNDs 3–6) was unaffected, while slant board performance (PNDs 8–11) was significantly poorer at the highest dose. Brains of culled pups (PND 1) showed dose-dependent increases of iAs. There were no significant AsV-related effects on PND 21 brain regional concentrations of dopamine, DOPAC, HVA, 5-HT or 5-HIAA. These hazard identification results will guide the study designs of developmental iAs exposure at human-relevant levels essential for risk-assessment.

1. Introduction

Arsenic is a naturally occurring element found ubiquitously throughout the environment and exists in inorganic and organic forms. The inorganic arsenics are thought to be particularly harmful to human health (ATSDR, 2007). The main sources of human exposure to inorganic arsenic (iAs) are food and water (ATSDR, 2007; WHO, 2011). iAs exposure through food, particularly rice and rice products, is of increasing concern to U.S. and European regulatory bodies (European Commission, 2015; FDA, 2016a; Karagas et al., 2016).

Rice accumulates more arsenic than other cereal crops because of its efficient nutrient transport pathways and anaerobic growing conditions (Liu et al., 2006; Williams et al., 2007; Zhao et al., 2010). In the U.S., approximately 90% of pregnant women eat rice or rice products (FDA, 2016a) and infant rice cereal is a common first food in the U.S. and abroad (Karagas et al., 2016; Inoue and Binns, 2014; Mennella et al., 2006; O'Donovan et al., 2015). It was recently estimated that 80% of infants are introduced to rice cereal in the first year, and urinary arsenic concentrations among infants eating infant rice cereal or snacks are up to three times higher than those who did not (Karagas et al., 2016).

The European Commission adopted a maximum limit of 100 ppb of arsenic for foodstuffs intended for infants and children in 2015 (European Commission, 2015; Karagas et al., 2016) and in the U.S., a similar limit is under regulatory consideration (FDA, 2016b). The FDA published a risk assessment in 2016 that included the potential health risks of exposure to iAs from rice and rice products in pregnancy through early childhood. Non-cancer neurotoxic effects were qualitatively identified, but a quantitative assessment was impeded by a lack

* Corresponding author.
E-mail address: Sherry.Ferguson@fda.hhs.gov (S.A. Ferguson).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.ntt.2019.01.007
Received 28 September 2018; Received in revised form 7 December 2018; Accepted 31 January 2019
Available online 06 February 2019
0892-0362/ Published by Elsevier Inc.

of available data (FDA, 2016a).

iAs is a known carcinogen and high level exposure is associated with cardiovascular toxicity and diabetes (Longnecker and Daniels, 2001; Moon et al., 2012). Much less clear are the effects of low level exposure during development (FDA, 2016a; NRC, 2013). Epidemiological studies suggest that low levels of arsenic exposure in utero and during early life may disrupt fetal growth and neurodevelopment (Fei et al., 2013; Wasserman et al., 2014). Due to their smaller body mass, infants and children are exposed to higher levels of contaminants. For example, exposure estimates of intake for infants are 0.03–1.6 μg/kg/day of iAs while similar exposure estimates for adults are 0.09–0.38 μg/kg/day (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009).

An international meta-analysis of studies of children exposed to high levels of arsenic indicated that a 50% increase in urinary arsenic levels was associated with a 0.4-point decrease in IQ (Rodriguez-Barranco et al., 2013). At lower levels such as those common in the U.S., there are reports of significant adverse effects of arsenic exposure. Children exposed to an average level of arsenic in drinking water of 9.88 μg/L exhibited a significant IQ decrease relative to children exposed to a lower level (Wasserman et al., 2014). That level (i.e., 9.88 μg/L) is below EPA's current maximum contaminant level (MCL) of 10 μg/L. Although a review of low level exposure studies concluded that there was only weak evidence to support a causative association of low level arsenic exposure and neurodevelopmental effects, that conclusion appeared to be based more on the number of poor quality studies that were excluded from the analysis and the few studies of good quality that were included (Tsuji et al., 2015).

Laboratory rodent studies of developmental iAs exposure have described neurobehavioral alterations. Such exposure has been shown to result in alterations of developmental milestones, righting reflex, cliff avoidance, and slant board behavior in most (Gumilar et al., 2015; Luo et al., 2013; Xi et al., 2009), but not all studies (Rodriguez et al., 2002). Learning deficits have also been reported in multiple tasks (Xi et al., 2009; Rodriguez et al., 2002). Many of those alterations occurred at high arsenic exposure levels administered via drinking water. In one study of relatively lower doses, female offspring of Wistar rats exposed to drinking water containing 50 or 100 μg/L from GD 0 through offspring PND 21 were delayed in performance of righting reflex and cliff aversion, while only male offspring of the higher dose group were delayed in performance of cliff aversion and slant board behavior (Gumilar et al., 2015). At adulthood, all arsenic-exposed females were hypoactive, but only males of the higher dose group were hypoactive. Those effects were striking given the lack of significant effects of exposure on gestational body weights, gestational length, litter size, or offspring body weights and possibly suggest a sex-specific effect of developmental arsenic exposure.

Very little arsenic is transferred via lactation in humans or rodents (Concha et al., 1998; Kozul-Horvath et al., 2012), yet there appears to be only one rodent study which directly exposed the offspring (Nagaraja and Desiraju, 1994). Here, developmental milestones, behavior, and neurochemical profiles of Sprague-Dawley rats exposed to iAs in utero through the dam's drinking water and then by direct dosing of the offspring by gavage from the day after birth (PND 1) through PND 21 were evaluated. Body weights and food and water intake were measured daily throughout. This pilot research was designed as a hazard identification study, not a full risk assessment. The results are intended to guide the design of future studies of developmental iAs exposure essential for appropriate hazard identification and risk assessment.

2. Materials and methods

2.1. Subjects and housing

Plug-positive timed pregnant Sprague-Dawley rats (n = 28) were received from Charles River (CD (SD) IGS Rat, strain code 001) on GD 5 in two shipments of 14 rats each. Each shipment was separated by three weeks. Rats were individually housed in standard polycarbonate cages with hardwood chip bedding. Cages were changed twice weekly. All had ad lib access to food (see below) and water in a vivarium with a 12/12 h light/dark schedule (lights on at 0600) and temperature of 22 ± 1 °C and 40–60% humidity with 10–15 air changes/h. Each rat was weighed daily throughout gestation and lactation. The NCTR is AAALAC accredited and all animal procedures were approved in advance by the NCTR Institutional Animal Care and Use Committee and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

On the day after parturition, litters were randomly culled to 8 (4/sex) and retained pups were paw tattooed for identification. Retained pups remained with their dam until the end of the study. All pups/litter were weighed daily. Any dam that had not littered by what would have been GD 26 was humanely euthanized.

2.2. Feed

Typical laboratory rodent chows contain measurable levels of iAs (Murko et al., 2018; Twaddle et al., 2018a). Here, rats consumed irradiated purified diet AIN-93G (TD.97184, Envigo, Madison, WI) which is reported to contain 10–15 ppb of iAs with no measurable monomethylarsonic acid (MMA^V) or dimethylarsinic acid (DMA^V) (Murko et al., 2018; Twaddle et al., 2018a). This diet has been suggested for developmental arsenic exposure studies of rodents (Murko et al., 2018). A small amount of this feed was delivered to the Charles River lab (Raleigh, NC) and those females that were plug positive were placed on this diet the morning after breeding. Thus, the low arsenic diet began at GD 0 and continued throughout. Food consumption was measured daily beginning at GD 5.

2.3. Water

Four weeks prior to the start of the study, samples of the normal rodent Millipore-filtered drinking water at the NCTR were assayed for 14 metals, including arsenic. Assays were done via inductively coupled plasma-atomic emission spectrometry (ICP-AES) by the Environmental Enterprise Group, Inc. (Russellville, AR). Total arsenic levels (inorganic and organic) were below the reporting limit (RL) of 0.05 ppm (i.e., the lowest concentration that can be quantified with a reasonable degree of statistical accuracy and precision). Of the other 13 metals assayed, only 4 were above the RL. Barium and copper levels, although detectable, were below the EPA's MCL for drinking water. The iron level was 0.15 ppm which is below the EPA's secondary MCL of 0.30 ppm. The manganese level was 0.021 ppm which is also below the EPA's secondary MCL of 0.05 ppm. Iron and manganese do not have primary MCLs.

2.4. Arsenic

Sodium arsenate dibasic heptahydrate (AsV) was purchased from Sigma Aldrich (St. Louis, MO) (catalog # A6756) and the purity was ≥98.0% as indicated by the Certificate of Analysis for the lot number.

2.5. Sample preparation

AsV was mixed with the NCTR normal Millipore-filtered rodent drinking water to formulate the desired concentrations (23.6, 47.4, or 71.0 ppm). AsV solutions were mixed weekly. Water bottles were weighed daily to determine intake. The concentrations of the arsenate in the drinking water and gavage formulations were determined following the reduction and derivatization procedures outlined in Minakata et al. (2009). Briefly, the drinking water formulation samples were diluted to a concentration of 0.236 μg/mL, while the gavage formulation samples were diluted to 23.6 μg/mL with Optima water

(Fisher Scientific, Hampton, NH). Calibrants at each level were then prepared in Optima water. 500 µL of the previously diluted water samples were added to a 2 mL centrifuge tube, along with 50 µL of 1 M hydrochloric acid (Fisher Scientific, Hampton, NH) and 50 µL of 0.1 M sodium thiosulfate (Sigma-Aldrich, St. Louis, MO). All samples were prepared in quintuplicate. Samples were briefly vortexed and allowed to reduce at room temperature for 5 min. Complexation of the arsenic was then achieved by adding 50 µL of 1 M sodium acetate (Sigma-Aldrich, St. Louis, MO) and 50 µL of 0.1 M pyrrolidinedithiocarbamate (Sigma-Aldrich, St. Louis, MO). Samples were vortexed for 10 s and diluted 1:50 with Optima water for LC/MS analysis.

2.6. Sample analysis

Dose certifications for the drinking and gavage water formulations were performed using a Waters Acquity I-class UPLC system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer equipped with an electrospray interface operating in positive ion mode (Waters Corporation, Milford, MA). Separation was achieved using a Waters BEH C18 column (2.1 × 50 mm, 1.7 µm). The column was held at 45 °C, while the sample chamber was maintained at 20 °C. Mobile phase A was 15 mM ammonium acetate, while mobile phase B was acetonitrile. Gradient conditions were as follows: initial conditions at 5% B; 5% B held for 1 min; 5–90% B in 1 min; 90% B held for 0.5 min; return to 5% B in 0.1 min. Total run time was 3.6 min and the injection volume was 1 µL. All samples were injected in triplicate. Single ion recording (SIR) detection at mass 367.10 was used for all samples and calibrants. The cone voltage was 6 V and the dwell time was 0.5 s. The accuracy of the dosing formulations (drinking water and gavage water) was then determined by comparing the peak areas for the dosed samples to those of the calibrant samples.

2.7. Arsenic treatment

Upon arrival at the NCTR on GD 5, each rat was weighed and assigned to a treatment group based on body weight such that each group had approximately the same starting average weight. Beginning the morning of GD 6, the regular water bottles were replaced with a water bottle containing 0, 23.6, 47.4, or 71.0 ppm AsV (n = 7/group). Water bottles were changed twice weekly. On the day of parturition, all water bottles were replaced with control water bottles (i.e., untreated rodent drinking water). Fig. 1 shows the experimental design.

Direct dosing by gavage of the offspring between PND 1 and 21 was necessary as very little arsenic is transferred via lactation in laboratory rodents or humans (Concha et al., 1998; Kozul-Horvath et al., 2012). Beginning on PND 1 (the day after parturition), all pups/litter were dosed by gavage with 0, 2.33, 4.67, or 7.00 mg/kg AsV in a volume of 1 mL/kg. Gavage solutions were 0, 2.33, 4.67, or 7.00 mg/mL. Dosing occurred daily between 8:00 and 9:00 am.

2.8. Arsenic species and dose justification

There are many more studies of developmental AsIII (arsenite) exposure than of AsV. However, although AsIII is stable in RO water, it is not stable in the normal Millipore-filtered rodent drinking water (Twaddle et al., 2018b). Thus, some AsIII in the normal drinking water would be converted to AsV and the exact ratio of AsIII to AsV would be uncertain. This could present a substantial confound with regard to exact dose and species ingested. We chose not to use a different type of drinking water (i.e., RO, distilled, or acidified) to maintain stability as there are health effects of long-term consumption of such waters (Kozisek, 2006; Hall et al., 1980; Wolf et al., 2014). Instead, AsV was the iAs species selected for use in this study.

AsV is also less toxic than AsIII (Vega et al., 2001; Stump et al., 1999; Souza et al., 2016; Lima et al., 2018) making the doses described in studies of AsIII not appropriate for comparison. We relied on 7 potentially relevant studies in the literature. Rats gavaged with 5 mg/kg AsV on PNDs 2–60 exhibited body weight reductions and alterations in the acquisition of continuous reinforcement behavior (Nagaraja and Desiraju, 1994). Fertility, mating and litter outcomes in mice were not described as altered by 50–55 ppb AsV in drinking water (Tyler et al., 2015; Martinez-Finley et al., 2009) and 12 weeks of exposure to 50 ppm AsV in drinking water did not produce adverse effects in female mice (Kenyon et al., 2008). After 10 consecutive days, mice gavaged daily with 0.5 mg AsV/kg did not exhibit any adverse health effects (Hughes et al., 2010). Two consecutive daily gavages on GD 7.5 and 8.5 of up to 14.4 mg/kg of AsV in mice did not alter gestational body weight but did decrease fetal weights (Hill et al., 2008). Finally, exposure to 100 ppm AsV in drinking water did not alter body weights of female rats (Adair et al., 2007). Our selection of 2.33, 4.67, and 7.00 mg/kg was based on this scant literature of AsV exposure. The desired ppm concentrations in the dams' drinking water were calculated based on historical records of average gestational water intake by Sprague-Dawley rats in our laboratory (Ferguson et al., 2011; Panos et al., 2014). Further, as this study was designed for hazard identification, not risk assessment, the higher doses are appropriate.

Finally, direct dosing of pups was necessary as rat pups are born at an earlier stage of development than humans (see for example, Ohmura

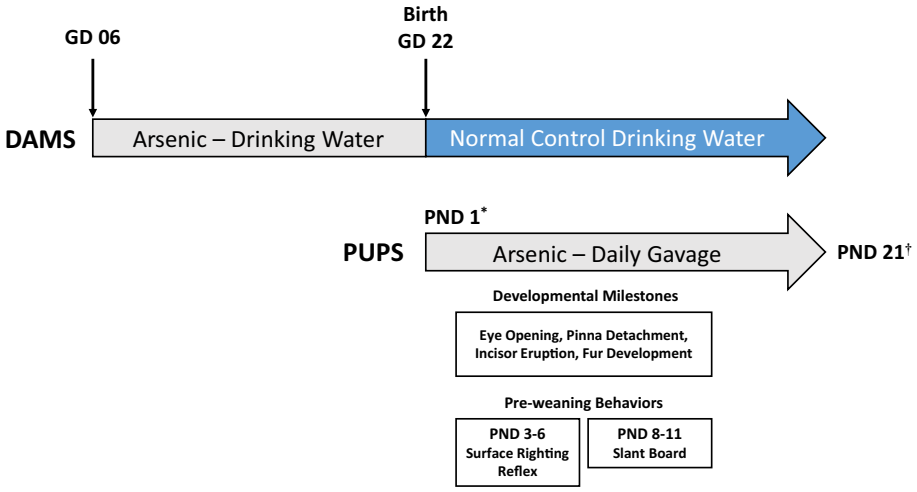

Fig. 1. Study schematic. Plug-positive timed pregnant Sprague-Dawley rats (n = 28) were divided into 4 groups and given either regular drinking water or arsenate (AsV; 23.6, 47.7 or 71.0 ppm) via drinking water beginning on gestational day 6 (GD 6) though GD 22. Dams were switched to regular drinking water at parturition. Dams were maintained on a low arsenic chow (AIN-93G) from GD 1 through offspring postnatal day 21 (PND 21). Litters were culled to 8 pups (4/sex/litter) on the day after parturition. Culled pup brains were analyzed for total inorganic arsenic content (*). The remaining pups were orally gavaged daily with the same arsenic treatment as their dams from PND 1 through the end of the study (PND 21). Age at full developmental landmark achievement was determined by daily observation. Righting reflex performance was tested daily on PNDs 3–6 and slant-board performance was tested daily on PNDs 8–11. On PND 21, frontal cortex, hippocampus and striatum were collected from all pups for HPLC analysis of neurotransmitter and metabolites levels (†).

and Kuniyoshi, 2017). The EPA and OECD guidelines for developmental neurotoxicity studies recommend dosing through lactational day 21 (see: U.S. EPA OPPTS 870.6300 and OECD 426 documents).

2.9. Endpoints

2.9.1. Body weight, food and water intake

Dams were weighed daily beginning on GD 5. Retained pups were selected randomly to ensure 4/sex/litter and were weighed daily beginning on PND 1. Food intake and water consumption were measured daily by weighing the feed hopper and water bottles.

2.9.2. Litter measures

Gestational length duration and number of total pups/litter were calculated on the day of birth (PND 0). On PND 1, sex ratio was calculated.

2.9.3. Arsenic content in culled pup brains

Culled pups were sacrificed by decapitation (n = 2–4/group and none were same-sex siblings) and brains quickly removed and frozen at −80 °C until analysis. Brains were assayed for oxidation state specific speciation which measured trivalent and pentavalent forms of iAs, MMA, and DMA. The method used was hydride generation-inductively-coupled-mass spectrometry with cryo-trapping (HG-CT-ICP-MS) (Currier et al., 2014). Assays were conducted under the supervision of Dr. Miroslav Styblo at the Atomic Absorption Spectrometry Lab of the Nutrition Obesity Research Center, Department of Nutrition, University of North Carolina (Chapel Hill, NC).

2.9.4. Developmental landmarks

All pups/litter were examined daily beginning on PND 7 for bilateral eye opening, bilateral ear opening, incisor eruption, and fur development. The PND at which each landmark was attained was recorded and the average PND/sex/litter was used in the statistical analyses. All examinations occurred prior to the daily gavage.

2.9.5. Surface righting reflex

On PNDs 3–6, all pups/litter were assessed for the righting reflex as previously described (Ferguson et al., 2010). Briefly, each pup was placed dorsal side down on a flat surface and the latency to right onto all four paws was recorded using a hand-held stopwatch (maximum latency 60 s). One trial/day was conducted by trained testers blind to treatment prior to the daily gavage. Latency to right was averaged by sex/litter for each PND prior to statistical analysis.

2.9.6. Slant board performance

On PNDs 8–11, all pups/litter were assessed for slant board behavior as previously described (Ferguson et al., 2010). Briefly, each pup was placed ventral side down on a sandpaper-covered board angled at 45° with its head pointed downward. Latency to turn 180° was measured using a hand-held stopwatch (maximum latency 60 s). Time of fall was recorded if the pup fell from the apparatus. One trial/day was conducted by trained testers blind to treatment prior to the daily gavage. Latency to turn was averaged by sex/litter for each PND prior to statistical analysis.

2.9.7. Whole and regional brain weights

At PND 21, 4/sex/group (randomly selected, but no same-sex siblings) were sacrificed via decapitation and the brain was quickly removed and weighed after which it was dissected into hippocampus, frontal cortex, and striatum as previously described (Ferguson et al., 1993). Those regions were weighed and frozen at −80 °C for later assays (see below).

2.9.8. Neurotransmitter and metabolite concentrations

Concentrations of dopamine (DA), serotonin (5-HT), and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (5-HIAA) were determined in the hippocampus, frontal cortex, and striatum of the frozen PND 21 brain regions via HPLC-EC as previously described (n = 4/sex/group, none were same-sex siblings) (Ferguson et al., 2005).

2.9.9. Statistical analysis

For bodyweights, food and water intake, righting reflex and slant board behavior, repeated measures ANOVAs were used to determine the effects of AsV treatment, sex (where included), and age or PND, as well as the interactions of these factors. Whole and regional brain weights and neurotransmitter and metabolite concentrations were analyzed by two-way ANOVAs with AsV treatment, sex, and the interaction as factors. Arsenic content in culled pup brains was analyzed with a two-way ANOVA with AsV treatment, sex, and the interaction as factors. For all analyses, $\alpha = 0.05$ and appropriate corrections for post hoc comparisons were made.

3. Results

3.1. Drinking water and gavage solution concentration analyses

Drinking water solutions ranged 95–103% of the intended concentrations and gavage solutions ranged 97–118% (n = 2/treatment). That high value was the result of one high dose (7.00 mg/ml) gavage solution that was assayed at 117% of intended concentration and a reassay of this same solution resulted in 118%. The other high dose gavage solution was 94.3% of intended concentration.

3.2. Body weight, food and water intake

Two control dams did not litter by what would have been GD 26. Their data were not included in any analyses. Analysis of gestational body weight indicated a significant interaction between AsV exposure and gestational day, F (48,352) = 2.09, $p < 0.001$; however, only one pairwise comparison was significantly different from the control group. The 71.0 ppm group weighed 8.6% less than the control group on GD 21 only ($p < 0.02$; Fig. 2A). During lactation, when dams were returned to untreated drinking water, there was no significant effect of prior AsV exposure ($F < 1.0$), but there was a significant main effect of lactational day (LD), F (20,440) = 17.95, $p < 0.001$. In general, body weights of all dams increased until LD 12, after which there was slight decrease after LD16 (Supplemental Fig. S1A).

During gestation, there was no significant effect of AsV exposure on food intake but there was a significant main effect of gestational day, F (14, 308) = 6.072, $p < 0.001$. Food intake varied day-to-day and there was no clear apparent pattern (Fig. 2B), but daily variation was generally < 3 g. During lactation, there was a significant AsV exposure by lactational day interaction, F (60, 440) = 1.454, $p = 0.02$. Food intake increased for all treatment groups throughout lactation and there were no significant pairwise comparisons except on LD 17 when dams in the 47.4 ppm exposure group consumed less food than control dams ($p < 0.001$; Fig. S1B). However, this effect is likely not biologically significant.

Analysis of water intake during gestation indicated a significant AsV exposure by gestational day interaction, F (45, 325) = 1.84, $p = 0.001$. Each of the three AsV exposed groups consumed significantly less water than the control group on all GDs (all p's < 0.04), except on GDs 7 and 17, when the 23.6 ppm group did not differ from control (Fig. 2C). During lactation, there was a trend toward a significant effect of AsV exposure, F (3, 437) = 2.70, $p = 0.07$, and a significant main effect of lactational day, F (20, 437) = 190.26, $p < 0.001$. Upon return to control drinking water, water consumption of all treatment groups returned to control levels, and water consumption generally increased throughout lactation (Fig. S1C).

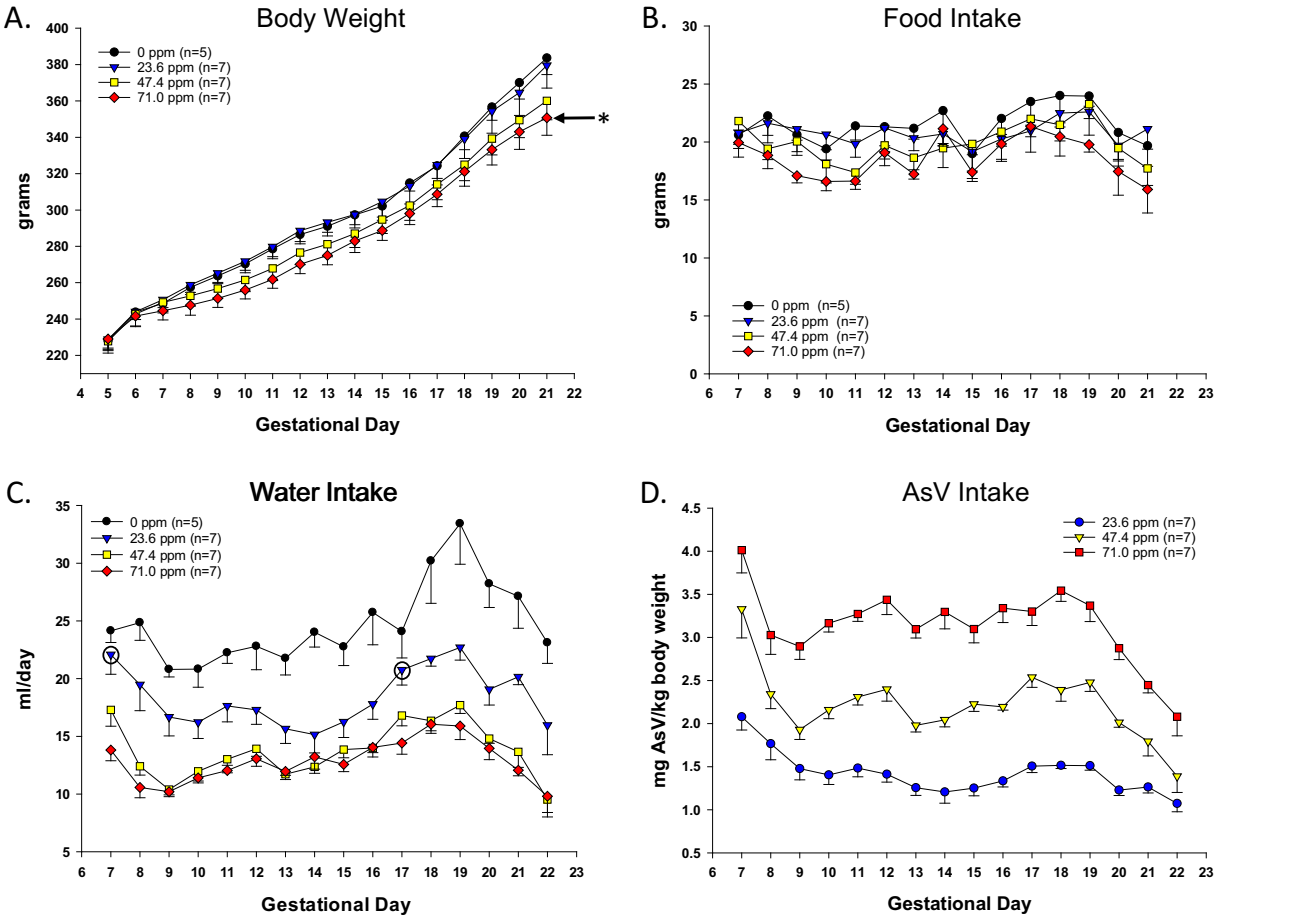

Fig. 2. Gestational measures. (A) Body weight, (B) food intake, (C) water intake (circled values are those that were not significantly different from control) and (D) calculated AsV intake of pregnant Sprague-Dawley rats from GDs 6–21. Body weight was significantly affected in the highest exposure group (71.0 ppm) on GD 22 only. *$p < 0.05$ compared to 0 ppm control.

3.3. Litter measures and pup bodyweight

There was no effect of AsV exposure on the total number of pups/litter, F (3, 22) = 1.26, $p = 0.31$ (M 11.29–13.20, SEM 0.52–0.92), or the sex ratio/litter, F (3, 22) = 1.25, $p = 0.32$ (M 0.40–0.56, SEM 0.13–0.17). There was no effect of AsV exposure on pup bodyweight, but there were significant main effects of sex, F (1, 44) = 5.21, $p = 0.03$, and postnatal day, F (20, 880) = 5991.47, $p < 0.001$, as well as a trend toward a PND by sex interaction, $F = 3.42$, $p = 0.06$. Those effects indicated that males weighed more than females and bodyweights increased with age (data not shown).

3.4. Arsenic content in pup brains

There was a significant effect of AsV exposure on the sum of iAs, MAs, and DMAs in the whole brain of PND 1 culled pups, F (3, 13) = 63.7, $p < 0.001$, but no significant effects of sex or a sex by AsV exposure interaction. Post-hoc comparisons indicated that brains of the 47.4 and 71.0 ppm exposure groups contained significantly more total As than brains of the control group (Table 1).

3.5. Developmental landmarks

There was no effect of AsV exposure, sex, or the interaction on fur development (F's < 1.80). The means ± SEM for age (PND) at fur development for control, low, medium and high AsV groups were 9.98 ± 0.24, 9.97 ± 0.20, 9.60 ± 0.19 and 9.80 ± 0.13, respectively. Likewise, there were no effects of AsV exposure, sex, or the interaction on bilateral ear opening (F's < 1.75). The means ± SEM for age (PND) at bilateral ear opening for control and AsV groups were 18.03 ± 0.03, 18.00 ± 0.00, 18.00 ± 0.00 and 18.00 ± 0.00, respectively. Analysis of age at bilateral eye opening, however, indicated a significant main effect of AsV exposure, F (3, 44) = 6.68, $p < 0.001$. Offspring in the three AsV exposed groups exhibited significant delays in eye opening which ranged from 0.60 day for the lowest AsV group to 1.12 days for the highest AsV group (p's < 0.02 for all comparisons to the control group; Fig. 3A). Similarly, there was a significant main effect of AsV exposure on incisor eruption, F (3, 44) = 3.88, $p = 0.02$. Offspring in the 47.4 ppm and 71.0 ppm exposure groups displayed delayed incisor eruption ranging from 0.61 to 0.70 day compared to the control group ($p < 0.05$ for comparisons to the control group); offspring exposed to 23.6 ppm AsV did not differ from control (see Fig. 3B).

3.6. Righting reflex

There were significant main effects of AsV exposure, sex, and PND on righting reflex behavior, F (3, 22) = 8.72, F (1, 22) = 11.93, and F (3, 66) = 37.51, respectively, all p's < 0.002. However, there were no significant interactions. Overall, offspring in the 71.0 ppm exposure group exhibited 25% longer righting latencies, but after correcting for multiple comparisons, this was no longer significant. As we have previously reported (Ferguson et al., 2011; Panos et al., 2014; Ferguson et al., 2010), males displayed shorter latencies and latencies decreased with age (Supplemental Fig. S2).

Table 1
Total arsenic in pup brains.

PND 1 culled pup brains			
Treatment (ppm)	Sample size (n)	Average total As (ppb ± SEM)	Raw values[a]
0.0	4	1.8 ± 0.1	**1.54**, **1.80**, 1.91, 2.05
23.6	4	377.1 ± 75.25	239.50, 314.10, **365.58**, **589.05**
47.4	2	1405.4 ± 372.28*	**1033.15**, **1777.63**
71.0	4	2968.6 ± 269.75*	**2337.38**, **2729.10**, **3265.82**, 3542.21

* $p < 0.05$.
[a] Bolded values are females.

3.7. Slant board performance

There was a significant main effect of AsV exposure on slant board behavior, F (3, 22) = 6.78, $p = 0.002$, and a significant main effect of PND, F (3, 66) = 13.687, $p < 0.001$. There was no significant effect of sex and no significant interactions. Generally, turning latencies decreased with age, but there was no significant difference between PND 9 and 10. To further describe the significant main effect of AsV exposure on slant board behavior, a Cox Proportional Hazards Model Survival Analysis was performed similar to that previously calculated for this endpoint (Ferguson et al., 2010). The analysis confirmed the significant effect of treatment (χ^2 (3) = 21.47, $p < 0.001$) with a greater probability of offspring in the 71.0 ppm exposure group not turning 180° within the time limit of 60 s relative to the control offspring. On the other hand, offspring in the 23.6 and 47.4 ppm exposure groups did not differ from control (Supplemental Fig. S3).

3.8. Whole and regional brain weights

There was no effect of AsV exposure on PND 21 weights of the whole brain, striatum, hippocampus, frontal cortex, or the brain to bodyweight ratio (Fs < 1.50). There was a significant effect of sex on whole brain weight, F (1, 24) = 6.04, $p = 0.02$, which indicated male brains weighed more than females, but that sex-related pattern was not apparent in the results of the analysis of the brain weight to bodyweight ratio. There were no other effects of sex or significant interactions on the weight of brain regions (Supplemental Table S1).

3.9. Neurotransmitters and metabolites

The levels of DA and DOPAC in the hippocampus and frontal cortex were below the limit of detection for several animals and were therefore not analyzed. For all other brain regions, there were no significant effects of AsV exposure, sex, or significant interactions on DA, DOPAC, 5-HT, 5-HIAA, or HVA in the frontal cortex, hippocampus or striatum at PND 21 (Fs < 2.19; Supplemental Table S2).

4. Discussion

Inorganic arsenic (iAs) is reported to affect neurodevelopment and cognition in rodents and humans at levels below those associated with acute toxicity and cancer (FDA, 2016a; Wasserman et al., 2014; Gumilar et al., 2015; Martinez-Finley et al., 2009; Tolins et al., 2014). As a more complete assessment of the risks of iAs exposure during gestation and infancy, Sprague-Dawley rats were exposed to AsV from gestation through weaning and assessed for developmental landmarks, preweaning behaviors and neurotransmitter levels. Although group sizes were relatively small in this preliminary study, AsV exposure produced striking effects. Food intake of the dams was not affected, but water intake was significantly reduced for all AsV concentrations during gestation. In their offspring, AsV exposure delayed incisor eruption and bilateral eye opening, while fur development and bilateral ear opening were unaffected. Righting reflex was not altered, while slant-board behavior was impaired at the high dose. Culled pup brains indicated significant iAs accumulation, but there were no significant effects on whole or regional brain weights or neurotransmitter levels in the striatum, frontal cortex or hippocampus. These results suggest iAs exposure during pregnancy and early development can act as a

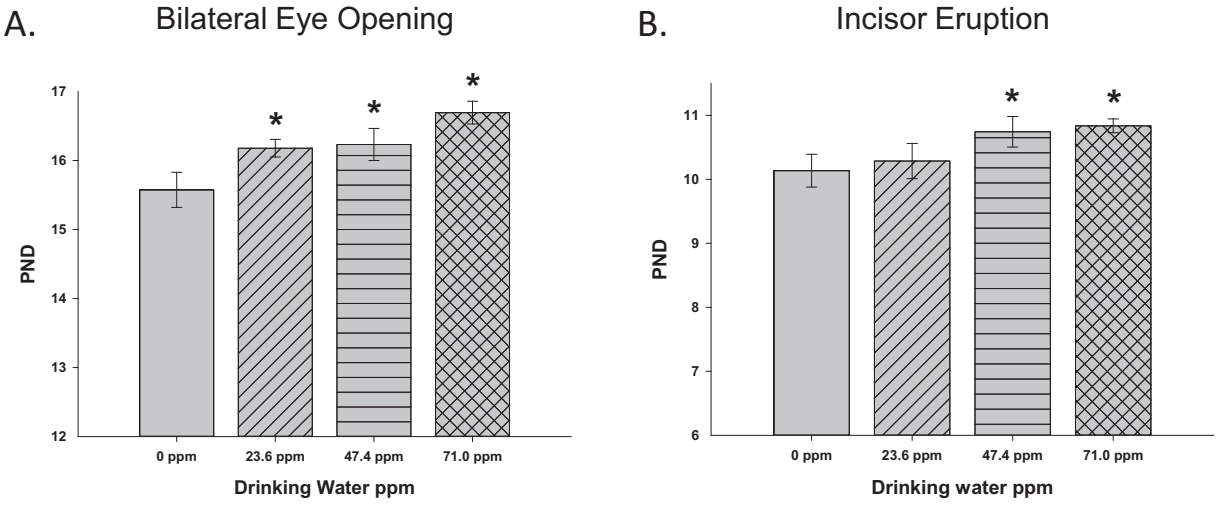

Fig. 3. Developmental landmarks. Average PND at which developmental landmarks were achieved by treatment group for (A) bilateral eye opening, (B) incisor eruption. Significant delays in eye opening occurred in all AsV groups, while significant delays in incisor eruption occurred in the 47.4 ppm and 71.1 ppm exposure groups. *$p < 0.05$ compared to 0 ppm control.

developmental neurotoxicant on early endpoints.

With one startling exception, there were few effects of AsV exposure on gestation, lactation and litter measures. Gestational food intake was unaffected. Gestational body weights were somewhat lower for the 47.4 and 71.0 ppm concentrations of AsV; however only the highest AsV group on GD 22 differed significantly from the control group. Although not statistically significant, a similar trend was described in Wistar rats exposed to AsIII (arsenite) (Xi et al., 2009). In the present study, there were no effects of prior iAs exposure on dam body weight or water intake during lactation and food intake indicated a single day difference from control which was likely due to an outlier. Litter measures including the total number of pups/litter, sex ratio/litter and pup body weights were unaffected by AsV exposure.

Although most gestational, lactational and litter measures were unaffected by AsV exposure, gestational water intake was strongly decreased by AsV concentration (Fig. 2C). Water intake was substantially reduced in all AsV groups (e.g., on GD 8, the 71.0 ppm dams consumed 57% less than control dams). The effect was immediate and sustained throughout gestation and somewhat dose-related. Whether this reduced drinking was a taste or odor aversion or a physiological effect is uncertain. Drinking water is often used as the method of iAs exposure in rodent studies (Gumilar et al., 2015; Luo et al., 2013; Xi et al., 2009; Rodriguez et al., 2002; Chandravanshi et al., 2014). Arsenic is known for being tasteless and odorless to humans and there are no reports of odor or taste aversion in rodents to arsenic in water. However, several reports have described a reduction of iAs water consumption by rodents (Xi et al., 2009; Fowler and Woods, 1979; Paul et al., 2007; Paul et al., 2011). Additionally, Garcia-Medina et al. (2007) paired iAs gavage with consumption of saccharin solutions which resulted in conditioned flavor aversion behavior in rats similar to that produced by lithium chloride (LiCl). An unpaired iAs and saccharin control experiment showed no effect of iAs on flavor response to saccharin, possibly indicating the decreased water intake here may be a physiological response rather than a taste aversion. On the other hand, the reduced water intake exhibited by arsenic methyltransferase (As3mt) knock-out mice relative to wild type mice was attributed to taste aversion. Specifically, water consumption was reduced by 50% across all doses (15, 20, 25, 30 ppm) while wild type C57BL/6 showed a similar decrease only at the highest concentration (Currier et al., 2016).

As a consequence of the decreased water intake in the present study, gestational AsV exposure was approximately half the intended target dose (1.42, 2.22, 3.14 mg/kg actual vs. 2.33, 4.67, 7.00 mg/kg intended). Interestingly, water intake at all concentrations of AsV recovered to control levels immediately upon the return to control water on the day after parturition which suggests a taste or odor aversion. Despite the lower than intended AsV exposure during gestation, culled pup brains taken at parturition (PND 1) showed significant accumulation of iAs, and the total arsenic in the pup brains was proportional to the dams' arsenic exposure in all treatment groups. In agreement with previous reports, arsenic amounts were dose-dependent and reflected the ability of iAs to easily cross the placental and blood-brain barriers (Xi et al., 2009; Rodriguez et al., 2002; Tolins et al., 2014; Xi et al., 2010).

Pups were dosed by gavage with AsV from PNDs 1–21 with the intended dam doses of 2.33, 4.67 and 7.00 mg/kg/day and observed for the achievement of physical developmental milestones. Age at fur development and bilateral ear opening were unaffected. Incisor eruption was delayed in the 47.7 and 71.0 ppm groups, while bilateral eye opening was delayed in all AsV groups. Few studies have evaluated the effect of arsenic on such endpoints, but among those, the results are somewhat inconsistent. For example, Colomina et al. (1997) reported that 10 mg/kg AsIII via gavage on GDs 15–18 delayed eye opening in female mice offspring. In Wistar rat offspring, continuous exposure to up to 100 ppm of AsIII in drinking water beginning at GD 6 had no effects on fur development, incisor eruption, or eye opening (Xi et al., 2009). On the other hand, Rodriguez et al. (2002) reported delays in bilateral ear and eye opening in Sprague-Dawley rats exposed to 36.7 ppm (approximately 2.93–4.20 mg/kg/day) of AsIII maternally from GD 15. There are no reports of the effects of AsV on similar landmarks. Delayed incisor eruption and bilateral eye opening may indicate endocrine disruptions (Gamborino et al., 2001; Silva et al., 2016). Arsenite accumulates in rat thyroid and influences thyroid levels of selenium and iodine (Kotyzova et al., 2005). Endocrine disruption of thyroid hormones can cause craniofacial malformations, including delayed postnatal eye opening in rats and delayed tooth eruption in rats and humans (Gamborino et al., 2001; Silva et al., 2016; Suri et al., 2004). While measuring endocrine and thyroid hormones was beyond the scope of this pilot study, it will be of value to examine those endpoints in future investigations of arsenic exposure.

The effect of AsV exposure on postnatal neurological reflexes and motor coordination were evaluated by performance of righting reflex on PNDs 3–6 and slant board behavior on PNDs 8–11. There was no effect of AsV treatment on righting reflex; however, the probability of not completing the 180° turn on the slant-board was significantly higher in the offspring of the 7.00 mg/kg group relative to control. There are no reports of AsV exposure on preweaning behaviors. However, the offspring of Sprague-Dawley dams drinking 13.5 ppm AsIII from GD 0 through LD 21 were impaired in performance of the righting reflex and slant board behavior (Luo et al., 2013). On the other hand, the female offspring of Wistar rats consuming 0.05 or 0.10 ppm AsIII during pregnancy and lactation were delayed in performance of the righting reflex while only males exposed to the higher concentration were impaired on slant board behavior (Gumilar et al., 2015). At first glance, this may indicate increased sensitivity of Wistar rats to the developmental effects of arsenic exposure and there is at least one report of murine strain differences in arsenic-induced hepatic injury (Wu et al., 2017). Deficits in these early behaviors may be predictive of cognitive deficits in later life. Future studies examining more complex behavior across the life span would be of great value.

There were no arsenic-related changes in whole brain, striatum, frontal cortex or hippocampus weights. Dose-dependent reductions in brain weights have been reported in prior developmental arsenic exposure studies (reviewed in Tolins et al., 2014) and arsenic exposure has been shown to alter dopamine and serotonin levels in specific brain regions (Mejia et al., 1997; Chandravanshi et al., 2018). However, there were no changes in dopamine, DOPAC, HVA, 5-HT or 5-HIAA levels in the present study. Results of the effects of arsenic on neurotransmitter concentrations are inconsistent, and the absence of effects in the current study supports the use of alternative indicators to global neurotransmitter concentrations in assessing the effects of arsenic exposure on neuronal function (Rodriguez et al., 2002; Rodriguez et al., 2001). In rodents, maternal and early life exposure to environmentally relevant levels of iAs has been shown to disrupt many molecular and biochemical functions in the brain (Tolins et al., 2014; Tyler and Allan, 2014). Those targets affect neuronal development and function through altered cell signaling, receptor dynamics, neurogenesis, stem cell differentiation, axonal growth, plasticity and apoptosis (Luo et al., 2013; Martinez-Finley et al., 2009; Rodriguez et al., 2001; Bain et al., 2016; Gilbert et al., 2016; Gilbert and Sui, 2006; Goggin et al., 2012; Luo et al., 2012; Pandey et al., 2017; Tyler and Allan, 2013). Future experiments looking at the effects of direct pup exposure on these processes could prove illuminating.

The current study had some potential limitations that should be acknowledged. The study was designed as a pilot experiment with relatively small group sizes of 5–7 litters per dose group and fewer subjects in the neurochemical analyses. Still, the significant arsenic-related effects are striking and other results (e.g., the significant sex effect on righting reflex performance) replicate our earlier studies. The overall results are somewhat difficult to integrate into the literature as AsV was used as a result of species stability issues with AsIII in drinking water.

While the present study has some potential limitations, it also has the advantage of several controls. The rodent drinking water and food

were assayed for levels of arsenic and the final sources provided (i.e., AIN-93G) were low in arsenic. The NCTR rodent drinking water was analyzed for arsenic and 13 other metals, including metals such as iron which have affinity for certain arsenic species and can render them insoluble and immobile. Here, the drinking water contribution to arsenic exposure was negligible. Typical laboratory rodent chow, such as NIH-41, is grain based and contains measurable levels of inorganic arsenic (Murko et al., 2018; Twaddle et al., 2018a). Here, rats consumed the irradiated purified diet AIN-93G which has 14–15 ppb of inorganic arsenic with no measurable MMA^V or DMA^V (Twaddle et al., 2018a). AsV was selected for use based on species stability in water. AsIII is not stable in normal Millipore-filtered drinking water as verified by in house stability analyses (Twaddle et al., 2018b). That instability was an important deciding factor to use AsV, although AsIII is a more toxic species and is commonly used in rodent studies. Finally, AsV was directly administered to offspring by gavage beginning on PND 1 to ensure precise exposure of the pups. Many developmental arsenic studies postnatally expose pups via lactation although arsenic is poorly transferred by this method.

The present study incorporated controls for arsenic exposure and direct gavage administration to offspring from the day after birth through PND 21 to understand the potential neurodevelopmental risks of arsenic exposure in pregnancy through weaning. All doses of AsV tested disrupted at least one measure of development or preweaning behavior suggesting these effects may occur at even lower exposure levels. Bilateral eye opening was disrupted at the lowest dose and may indicate some level of neurodevelopmental endocrine disruption that could affect adult cognitive abilities. The results of this pilot study will guide the design of future studies of developmental iAs exposure at human-relevant levels essential for appropriate hazard identification and risk assessment. Additionally, CFSAN (the FDA's Center for Food Safety and Nutrition) recently created a Toxic Elements Working Group to prioritize the Center's actions related to toxic elements in order to reduce exposure to consumers to the greatest extent feasible. The results of this study, as discussed in this paper, will help inform the strategy for toxic elements, such as arsenic, that are found in foods consumed by infants and children.

Funding

This work was supported by National Center for Toxicological Research protocol E0220601 of the U.S. Food & Drug Administration (FDA) funded by the National Toxicology Program under an Interagency Agreement between FDA and National Institute of Environmental Health Sciences (NIEHS) (FDA IAG #224-17-0502 and NIH IAG #AES12013). TJF was partially supported by an ORISE postdoctoral fellowship.

Disclaimer

This document has been reviewed in accordance with United States Food and Drug Administration (FDA) policy and approved for publication. Approval does not signify that the contents necessarily reflect the position or opinions of the FDA nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the FDA.

Conflicts of interest

The authors have nothing to declare.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

We appreciate the arsenic speciation testing performed by Drs. Christelle Douillet and Mirek Styblo with the Department of Nutrition at the University of North Carolina – Chapel Hill supported by National Institutes of Health Grant DK056350 to the UNC-CH Nutrition Obesity Research Center. The authors are grateful for the technical skills provided by the animal care staff of the Priority One Corporation and the expertise of Ms. Bonnie Robinson.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ntt.2019.01.007.

References

Adair, B.M., et al., 2007. Tissue distribution and urinary excretion of dimethylated arsenic and its metabolites in dimethylarsinic acid- or arsenate-treated rats. Toxicol. Appl. Pharmacol. 222 (2), 235–242.
ATSDR, 2007. Toxicological Profile for Arsenic. (Atlanta, GA).
Bain, L.J., Liu, J.T., League, R.E., 2016. Arsenic inhibits stem cell differentiation by altering the interplay between the Wnt3a and Notch signaling pathways. Toxicol. Rep. 3, 405–413.
Chandravanshi, L.P., et al., 2014. Early life arsenic exposure and brain dopaminergic alterations in rats. Int. J. Dev. Neurosci. 38, 91–104.
Chandravanshi, L.P., Gupta, R., Shukla, R.K., 2018. Arsenic-induced neurotoxicity by dysfunctioning cholinergic and dopaminergic system in brain of developing rats. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-018-1452-5.
Colomina, M.T., et al., 1997. Influence of maternal stress on the effects of prenatal exposure to methylmercury and arsenic on postnatal development and behavior in mice: a preliminary evaluation. Physiol. Behav. 61 (3), 455–459.
Concha, G., et al., 1998. Low-level arsenic excretion in breast milk of native Andean women exposed to high levels of arsenic in the drinking water. Int. Arch. Occup. Environ. Health 71 (1), 42–46.
Currier, J.M., et al., 2014. Associations between arsenic species in exfoliated urothelial cells and prevalence of diabetes among residents of Chihuahua, Mexico. Environ. Health Perspect. 122 (10), 1088–1094.
Currier, J.M., et al., 2016. Oxidation state specific analysis of arsenic species in tissues of wild-type and arsenic (+3 oxidation state) methyltransferase-knockout mice. J. Environ. Sci. (China) 49, 104–112.
EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009. Scientific opinion on arsenic in food. EFSA J. 7 (10), 1351.
European Commission, 2015. Commission Regulation (EU) 2015/2016 of 25 June 2015 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Inoragnic Arsenic in Foodstuffs.
FDA, FDA (Eds.), 2016. Arsenic in Rice and Rice Products Risk Assessment Report.
FDA, 2016b. FDA Proposes Limit for Inorganic Arsenic in Infant Cereal.
Fei, D.L., et al., 2013. Association between in utero arsenic exposure, placental gene expression, and infant birth weight: a US birth cohort study. Environ. Health 12, 58.
Ferguson, S.A., et al., 1993. Behavioral effects of methylazoxymethanol-induced micrencephaly. Behav. Neurosci. 107 (6), 1067–1076.
Ferguson, S.A., et al., 2005. Four weeks of oral isotretinoin treatment causes few signs of general toxicity in male and female Sprague-Dawley rats. Food Chem. Toxicol. 43 (8), 1289–1296.
Ferguson, S.A., et al., 2010. Preweaning behaviors, developmental landmarks, and acrylamide and glycidamide levels after pre- and postnatal acrylamide treatment in rats. Neurotoxicol. Teratol. 32 (3), 373–382.
Ferguson, S.A., Law Jr., C.D., Abshire, J.S., 2011. Developmental treatment with bisphenol A or ethinyl estradiol causes few alterations on early preweaning measures. Toxicol. Sci. 124 (1), 149–160.
Fowler, B.A., Woods, J.S., 1979. The effects of prolonged oral arsenate exposure on liver mitochondria of mice: morphometric and biochemical studies. Toxicol. Appl. Pharmacol. 50 (2), 177–187.
Gamborino, M.J., et al., 2001. Role of thyroid hormone in craniofacial and eye development using a rat model. Ophthalmic Res. 33 (5), 283–291.
Garcia-Medina, N.E., et al., 2007. Conditioned flavor aversion and brain Fos expression following exposure to arsenic. Toxicology 235 (1–2), 73–82.
Gilbert, M.E., Sui, L., 2006. Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. Brain Res. 1069 (1), 10–22.
Gilbert, M.E., Sanchez-Huerta, K., Wood, C., 2016. Mild thyroid hormone insufficiency during development compromises activity-dependent neuroplasticity in the hippocampus of adult male rats. Endocrinology 157 (2), 774–787.
Goggin, S.L., Labrecque, M.T., Allan, A.M., 2012. Perinatal exposure to 50 ppb sodium arsenate induces hypothalamic-pituitary-adrenal axis dysregulation in male C57BL/6 mice. Neurotoxicology 33 (5), 1338–1345.
Gumilar, F., et al., 2015. Locomotor activity and sensory-motor developmental alterations in rat offspring exposed to arsenic prenatally and via lactation. Neurotoxicol. Teratol. 49, 1–9.

Hall, J.E., White, W.J., Lang, C.M., 1980. Acidification of drinking water: its effects on selected biologic phenomena in male mice. Lab. Anim. Sci. 30 (4 Pt 1), 643–651.
Hill, D.S., Wlodarczyk, B.J., Finnell, R.H., 2008. Reproductive consequences of oral arsenate exposure during pregnancy in a mouse model. Birth Defects Res. B Dev. Reprod. Toxicol. 83 (1), 40–47.
Hughes, M.F., et al., 2010. Arsenic (+3 oxidation state) methyltransferase genotype affects steady-state distribution and clearance of arsenic in arsenate-treated mice. Toxicol. Appl. Pharmacol. 249 (3), 217–223.
Inoue, M., Binns, C.W., 2014. Introducing solid foods to infants in the Asia Pacific region. Nutrients 6 (1), 276–288.
Karagas, M.R., et al., 2016. Association of rice and rice-product consumption with arsenic exposure early in life. JAMA Pediatr. 170 (6), 609–616.
Kenyon, E.M., et al., 2008. Tissue distribution and urinary excretion of inorganic arsenic and its methylated metabolites in C57BL6 mice following subchronic exposure to arsenate in drinking water. Toxicol. Appl. Pharmacol. 232 (3), 448–455.
Kotyzova, D., et al., 2005. Effect of long-term administration of arsenic (III) and bromine with and without selenium and iodine supplementation on the element level in the thyroid of rat. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 149 (2), 329–333.
Kozisek, F., 2006. Health effects of long term consumption of water low in calcium, magnesium or TDS: studies from Eastern Europe. In: International Symposium on Health Aspects of Calcium and Magnesium in Drinking Water. NSF International, Baltimore, MD.
Kozul-Horvath, C.D., et al., 2012. Effects of low-dose drinking water arsenic on mouse fetal and postnatal growth and development. PLoS One 7 (5), e38249.
Lima, G.D.A., et al., 2018. Fertility in male rats: disentangling adverse effects of arsenic compounds. Reprod. Toxicol. 78, 130–140.
Liu, W.J., et al., 2006. Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (Oryza sativa L.). Environ. Sci. Technol. 40 (18), 5730–5736.
Longnecker, M.P., Daniels, J.L., 2001. Environmental contaminants as etiologic factors for diabetes. Environ. Health Perspect. 109 (Suppl. 6), 871–876.
Luo, J.H., et al., 2012. Arsenite exposure altered the expression of NMDA receptor and postsynaptic signaling proteins in rat hippocampus. Toxicol. Lett. 211 (1), 39–44.
Luo, J., et al., 2013. Maternal and early life arsenite exposure impairs neurodevelopment and increases the expression of PSA-NCAM in hippocampus of rat offspring. Toxicology 311 (3), 99–106.
Martinez-Finley, E.J., Ali, A.M., Allan, A.M., 2009. Learning deficits in C57BL/6J mice following perinatal arsenic exposure: consequence of lower corticosterone receptor levels? Pharmacol. Biochem. Behav. 94 (2), 271–277.
Mejia, J.J., et al., 1997. Effects of lead-arsenic combined exposure on central monoaminergic systems. Neurotoxicol. Teratol. 19 (6), 489–497.
Mennella, J.A., et al., 2006. Feeding Infants and Toddlers Study: the types of foods fed to hispanic infants and toddlers. J. Am. Diet. Assoc. 106 (1), S96–S106.
Minakata, K., Suzuki, M., Suzuki, O., 2009. Simple and selective determination of arsenite and arsenate by electrospray ionization mass spectrometry. Anal. Chim. Acta 631 (1), 87–90.
Moon, K., Guallar, E., Navas-Acien, A., 2012. Arsenic exposure and cardiovascular disease: an updated systematic review. Curr. Atheroscler. Rep. 14 (6), 542–555.
Murko, M., et al., 2018. Dose and diet - sources of arsenic intake in mouse in utero exposure scenarios. Chem. Res. Toxicol. 31, 156–164.
Nagaraja, T.N., Desiraju, T., 1994. Effects on operant learning and brain acetylcholine esterase activity in rats following chronic inorganic arsenic intake. Hum. Exp. Toxicol. 13 (5), 353–356.
NRC, 2013. Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report. National Research Council.
O'Donovan, S.M., et al., 2015. Adherence with early infant feeding and complementary feeding guidelines in the Cork BASELINE Birth Cohort Study. Public Health Nutr. 18 (15), 2864–2873.
Ohmura, Y., Kuniyoshi, Y., 2017. A translational model to determine rodent's age from human foetal age. Sci. Rep. 7 (1), 17248.
Pandey, R., et al., 2017. From the cover: arsenic induces hippocampal neuronal apoptosis and cognitive impairments via an up-regulated BMP2/Smad-dependent reduced BDNF/TrkB signaling in rats. Toxicol. Sci. 159 (1), 137–158.
Panos, J.J., Law, C.D., Ferguson, S.A., 2014. Effects of perinatal methylphenidate (MPH) treatment in male and female Sprague-Dawley offspring. Neurotoxicol. Teratol. 42C, 9–16.
Paul, D.S., et al., 2007. Examination of the effects of arsenic on glucose homeostasis in cell culture and animal studies: development of a mouse model for arsenic-induced diabetes. Toxicol. Appl. Pharmacol. 222 (3), 305–314.
Paul, D.S., et al., 2011. Characterization of the impaired glucose homeostasis produced in C57BL/6 mice by chronic exposure to arsenic and high-fat diet. Environ. Health Perspect. 119 (8), 1104–1109.
Rodriguez, V.M., et al., 2001. The effects of sodium arsenite exposure on behavioral parameters in the rat. Brain Res. Bull. 55 (2), 301–308.
Rodriguez, V.M., et al., 2002. Effects of sodium arsenite exposure on development and behavior in the rat. Neurotoxicol. Teratol. 24 (6), 743–750.
Rodriguez-Barranco, M., et al., 2013. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci. Total Environ. 454–455, 562–577.
Silva, M.A., et al., 2016. Parathyroid hormone intermittent administration promotes delay on rat incisor eruption. Arch. Oral Biol. 69, 102–108.
Souza, A.C.F., et al., 2016. Effects of sodium arsenite and arsenate in testicular histomorphometry and antioxidants enzymes activities in rats. Biol. Trace Elem. Res. 171 (2), 354–362.
Stump, D.G., et al., 1999. Comparative effects of single intraperitoneal or oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology 60 (5), 283–291.
Suri, L., Gagari, E., Vastardis, H., 2004. Delayed tooth eruption: pathogenesis, diagnosis, and treatment. A literature review. Am. J. Orthod. Dentofac. Orthop. 126 (4), 432–445.
Tolins, M., Ruchirawat, M., Landrigan, P., 2014. The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. Ann. Glob. Health 80 (4), 303–314.
Tsuji, J.S., et al., 2015. Low-level arsenic exposure and developmental neurotoxicity in children: a systematic review and risk assessment. Toxicology 337, 91–107.
Twaddle, N.C., et al., 2018a. Metabolism and disposition of arsenic species from controlled oral dosing with sodium arsenite in adult female CD-1 mice. I. Pilot study to determine dosing, analytical measurements, and sampling strategies. Food Chem. Toxicol. 111, 482–493.
Twaddle, N.C., et al., 2018b. Metabolism and disposition of arsenic species after repeated oral dosing with sodium arsenite in drinking water. II. Measurements in pregnant and fetal CD-1 mice. Food Chem. Toxicol. 115, 178–184.
Tyler, C.R., Allan, A.M., 2013. Adult hippocampal neurogenesis and mRNA expression are altered by perinatal arsenic exposure in mice and restored by brief exposure to enrichment. PLoS One 8 (9), e73720.
Tyler, C.R., Allan, A.M., 2014. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr. Environ. Health Rep. 1, 132–147.
Tyler, C.R., et al., 2015. Developmental exposure to 50 parts-per-billion arsenic influences histone modifications and associated epigenetic machinery in a region- and sex-specific manner in the adult mouse brain. Toxicol. Appl. Pharmacol. 288 (1), 40–51.
Vega, L., et al., 2001. Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. Toxicol. Appl. Pharmacol. 172 (3), 225–232.
Wasserman, G.A., et al., 2014. A cross-sectional study of well water arsenic and child IQ in Maine schoolchildren. Environ. Health 13 (1), 23.
WHO, 2011. Safety evaluation of certain contaminants in food. In: WHO Food Aditive Series No. 63.
Williams, P.N., et al., 2007. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. Environ. Sci. Technol. 41 (19), 6854–6859.
Wolf, K.J., et al., 2014. Consumption of acidic water alters the gut microbiome and decreases the risk of diabetes in NOD mice. J. Histochem. Cytochem. 62 (4), 237–250.
Wu, R., et al., 2017. Strain differences in arsenic-induced oxidative lesion via arsenic biomethylation between C57BL/6J and 129X1/SvJ mice. Sci. Rep. 7, 44424.
Xi, S., et al., 2009. Transplacental and early life exposure to inorganic arsenic affected development and behavior in offspring rats. Arch. Toxicol. 83 (6), 549–556.
Xi, S., et al., 2010. Distribution and speciation of arsenic by transplacental and early life exposure to inorganic arsenic in offspring rats. Biol. Trace Elem. Res. 134 (1), 84–97.
Zhao, F.J., McGrath, S.P., Meharg, A.A., 2010. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annu. Rev. Plant Biol. 61, 535–559.