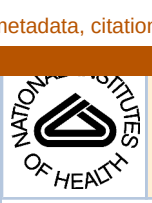

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Arsenicals in maternal and fetal mouse tissues after gestational exposure to arsenite

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Abstract

Exposure of pregnant C3H/HeNCR mice to 42.5- or 85-ppm of arsenic as sodium arsenite in drinking water between days 8 and 18 of gestation markedly increases tumor incidence in their offspring. In the work reported here, distribution of inorganic arsenic and its metabolites, methyl arsenic and dimethyl arsenic, were determined in maternal and fetal tissues collected on gestational day 18 of these exposure regimens. Tissues were collected from three females and from associated fetuses exposed to each dosage level. Concentrations of total speciated arsenic (sum of inorganic, methyl, and dimethyl arsenic) were higher in maternal tissues than in placenta and fetal tissues; total speciated arsenic concentration in placenta exceeded those in fetal tissues. Significant dosage-dependent (42.5 ppm versus 85 ppm of arsenite in drinking water) differences were found in total speciated arsenic concentrations in maternal lung ($p < 0.01$) and liver ($p < 0.001$). Total speciated arsenic concentrations did not differ significantly between dosage levels for maternal blood or for fetal lung, liver, and blood, or for placenta. Percentages of inorganic, methyl, or dimethyl arsenic in maternal or fetal tissues were not dosage-dependent. Over the range of total speciated arsenic concentrations in most maternal and fetal tissues, dimethyl arsenic was the most abundant arsenical. However, in maternal liver at the highest total speciated arsenic concentration, inorganic arsenic was the most abundant arsenical, suggesting that a high tissue burden of arsenic affected formation or retention of methylated species in this organ. Tissue concentration-dependent processes could affect kinetics of transfer of inorganic arsenic or its metabolites from mother to fetus.

Keywords

Arsenic; Mouse; *In utero*; Metabolism; Transplacental carcinogenesis

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1. Introduction

Exposure to inorganic arsenic (iAs) has been associated with developmental and reproductive toxicity (Willhite and Ferm, 1984), although such effects are dependent upon the route of exposure (Holson et al., 2000). Because iAs is rapidly metabolized to methylated forms, attribution of a particular adverse effect to a single arsenical species is problematic. In experimental species, maternal exposure to inorganic arsenic (iAs) resulted in fetal exposure to iAs and its metabolites, methyl arsenic (MAs) and dimethyl arsenic (DMAs) (Tanaka, 1976; Lindgren et al., 1982; Hood et al., 1987,1988). Exposure to MAs or DMAs produced maternal and fetal toxicity and teratogenesis in rodents (Rogers et al., 1981; Hood, 1998), suggesting that these arsenicals or their metabolites are the active forms. In women chronically consuming iAs-contaminated drinking water during pregnancy, DMAs was the predominant arsenical in either maternal or cord plasma (Concha et al., 1998); thus, iAs or its metabolites must cross human placenta. Although evidence of teratogenesis is lacking in iAs-exposed humans, reproductive toxicity has been reported in populations with high levels of exposure to iAs. Chakraborti et al. (2003) found increased incidences of miscarriages, stillbirths, preterm births, and low birth weights in offspring of women who used drinking water containing 463–1025 μg iAs per l. Other studies of reproductive outcome in populations with high levels of iAs exposure have revealed increased rates of spontaneous abortion and of perinatal and neonatal mortality (Hopenhayn-Rich et al., 1998; Ahmad et al., 2001). Short and long-term effects of transplacental exposure to iAs in humans have been reported. Ingestion of large doses of iAs during pregnancy has been linked to intrauterine fetal death (Bolliger et al., 1992) and to death of a premature infant (Lugo et al., 1969). The occurrence of multiple basilomata in a young man has been associated with therapeutic use of Fowler's solution (potassium arsenite) by his mother during pregnancy (Aldick and Fabry, 1973).

Prominent among adverse health effects associated with chronic exposure to iAs in either occupational or environmental settings is increased risk of cancer. An IARC review concluded that there was sufficient evidence that arsenic in drinking water causes cancer of urinary bladder, lung, and skin in exposed humans (IARC, 2004). By comparison, this review concluded that there was only limited evidence that arsenite (iAs^{III}) was a carcinogen in experimental species. Indeed, activity of iAs^{III} as a tumor promoter or as a carcinogen has been demonstrated only in genetically modified animals or in animal models for co-carcinogenicity (Germolec et al., 1998; Rossman, 2003). In contrast, exposure of fetal mice to iAs^{III} increases cancer risk in later life. Apparently healthy offspring of C3H female mice that consumed drinking water containing 42.5- or 85-ppm iAs^{III} between gestational days 8–18 developed tumors in adult life (Waalkes et al., 2003,2004a). Tumor sites differed markedly between males and females exposed *in utero* to iAs^{III}. Males showed dose-related increases in the number and multiplicity of hepatocellular carcinomas; females showed dose-related increases in the number and multiplicity of lung and ovary tumors. In addition, postnatal treatment with phorbol ester in mice exposed *in utero* to iAs^{III} increased lung tumor yield in both sexes and liver tumor yield in females (Waalkes et al., 2004b). In females exposed *in utero* to iAs^{III}, postnatal exposure to diethylstilbestrol or tamoxifen increased the yield of uroepithelial proliferative lesions or urogenital tumors (Waalkes et al., 2006). Hence, iAs^{III} is a complete transplacental carcinogen and a tumor initiator in the fetal mouse.

Conversion of iAs to methylated metabolites is an enzymatically catalyzed process in which oxidative methylation reactions using *S*-adenosylmethionine (AdoMet) as a methyl group donor alternates with reactions in which arsenic is reduced from pentavalency to trivalency (Cullen et al., 1984; Thomas et al., 2004). A single enzyme, arsenic (+3 oxidation state) methyltransferase (AS3MT), plays a central role in the metabolic transformation of iAs to methylated products (Lin et al., 2002). Because some intermediates and products (e.g., MAs^{III} and $DMAs^{III}$) formed in this metabolic pathway are more reactive and toxic than

iAs^{III}, methylation of iAs is an activation process (Thomas et al., 2001). Thus, it is possible that some of the adverse effects associated with *in utero* exposure to iAs^{III} could be mediated by actions of iAs or by one of its reactive metabolites. In the work reported here, we have quantified arsenicals in maternal and fetal tissues at the end of a regimen of exposure *in utero* to iAs that has been shown to produce tumor development in adult life. Although concentrations of arsenicals in maternal tissues and placenta exceeded those in fetal tissues, relatively high concentrations of iAs, MAs, and DMAs were also present in fetal tissues. These findings suggest that translocation of arsenicals from mother to fetus and metabolism of iAs in fetal tissues may contribute to the accumulation of arsenicals in fetal tissues during critical periods of growth and development.

2. Materials and methods

2.1. Chemicals

All reagents were prepared in deionized water (18 MΩ/cm). Only analytical grade chemicals were used throughout the study, including sodium borohydride ($NaBH_4$, EM Science, Gibbstown, NJ), NaOH (EM Science), HCl (Fisher Chemicals, Fair Lawn, NJ), ultrapure phosphoric acid (J.T. Baker, Inc., Phillipsburg, NJ), and antifoam B silicone emulsion (J.T. Baker). ACS certified HNO_3, H_2SO_4, $HClO_4$, and HCl (all from EM Science) were used for total arsenic analysis. Pentavalent arsenical standards for analysis by hydride generation-atomic absorption spectrometry (HG-AAS) were arsenic acid, sodium salt (Sigma, St. Louis, MO), methylarsonic acid, disodium salt (Chem Service, West Chester, PA), and dimethylarsinic acid (Strem, Newburyport, MA). An AA/ICP solution containing 992 μg of arsenic per ml in 0.2% HNO_3 (Sigma–Aldrich) was the standard for total arsenic analysis by hydride generation-atomic fluorescence spectrometry (HG-AFS).

2.2. Animal husbandry and treatments

Timed pregnant C3H/HeNCR mice obtained from the Animal Production Area, NCI-Frederick (Frederick, MD) were housed in a standard barrier facility in a 12 h light/dark cycle at 68–72 F and a relative humidity of 50 ± 5%. Throughout treatment, mice had free access to basal diet (NIH-31 Open Formula, 6% modified, Teklad, Madison, WI). Between gestational days 8 and 18, pregnant mice had free access to drinking water containing 42.5- or 85-ppm arsenic as sodium arsenite (iAs^{III}). Addition of iAs^{III} at these dosage levels does not affect drinking water consumption or weight gain as compared to responses in pregnant mice receiving drinking water without added iAs^{III} (Waalkes et al., 2003). On gestational day 18, mice were euthanized by CO_2 asphyxiation and tissues collected for analysis of arsenic contents. Liver, lung, and blood were collected from each of three pregnant females. Placentas were pooled for analysis. Fetuses were processed to yield liver, lung, and blood samples. Blood was collected from decapitated fetuses. All tissues were snap frozen in liquid nitrogen and stored at −70 °C until analyzed.

2.3. Arsenic analysis

Speciated arsenicals in tissues were determined by HG-AAS as previously described (Devesa et al., 2004). Tissues were prepared for analysis by overnight digestion at 90 °C in 3 ml of 2 M ultrapure phosphoric acid (J.T. Baker). Arsines were generated at pH 1 from a reaction mixture containing 1% antifoam B silicone emulsion (1 ml), deionized water (7 ml), 6N HCl (1 ml), and an aliquot of the digested tissue by addition of 1 ml of 4% $NaBH_4$ in 0.02 M NaOH. Generated arsines were swept in a helium stream (200 ml per min) to a U-tube containing Chromosorb WAW-dimethyldichlorosilane 46/60 (Supelco, Inc., Bellefonte, PA) that was immersed in liquid nitrogen. After a 3-min collection period, the U-tube was transferred from liquid nitrogen to room temperature and warmed to allow boiling point-dependent release of arsines. Arsines were carried into a quartz atomization cuvette in a Perkin-Elmer model 5100

atomic absorption spectrometer. The cuvette was heated with an air (140 ml per min) and hydrogen (610 ml per min) flame. Under these conditions, this method routinely resolves three arsenicals (iAs, MAs, and DMAs). Four concentrations (2.5, 10, 20, 80 ng) of each of these arsenicals were used to prepare calibration curves. Arsenicals in tissue samples were identified by spiking samples with arsenical standards at several concentrations. Total speciated arsenic concentration in a tissue sample was the sum of concentrations of iAs, MAs, and DMAs. To assess recoveries of arsenicals in speciation analysis by HG-AAS, total arsenic contents of mouse tissues were determined by HG-AFS using an automated ProStar 410 autosampler and 9012 quaternary pump (Varian Analytical Instruments, Palo Alto, CA, USA) with a Millennium Excalibur HG-AFS system (PSAnalytical Ltd., Kent, England). HG-AFS analysis was performed as previously described (Ma and Le, 1998). Briefly, tissues were completely wet-digested by sequential addition of nitric and perchloric acid followed by sulfuric acid (Devesa et al., 2004). Aliquots of digestates (100 μl) were injected into the hydride generator in deionized water (3 ml per min), mixed with 5% HCl (1.9 ml per min), and then with 1.4% $NaBH_4$ (in 0.1 M NaOH, 1.9 ml per min). Arsines were carried in an Ar stream (235 ml per min) to a fluorescence detector (Stockwell and Corns, 1994). Recovery of arsenic from a tissue was calculated as total speciated arsenic concentration (determined by HG-AAS) divided by the total arsenic concentration (determined by HG-AFS).

2.4. Statistical analysis

Differences between tissue concentrations of total speciated arsenic or of concentrations of each of arsenic species in tissues of dams and fetuses in the two treatment groups were evaluated by one-way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison post-test. These analyses focused mainly on differences related to dosage level and on differences in the concentrations of arsenicals in corresponding maternal and fetal tissues. A GraphPad Instat software package (GraphPad Software Inc., San Diego, CA) was used for this analysis. Differences at the $p < 0.05$ level were considered statistically significant.

3. Results

3.1. Tissue distribution of arsenicals

Concentrations of iAs, MAs, and DMAs were determined by HG-AAS in maternal and fetal tissues collected on gestational day 18, at the end of a 10-day regimen of consumption of drinking water that contained 42.5- or 85-ppm iAs^{III}. Compared to results from total arsenic analysis by HG-AFS, recoveries of speciated arsenicals determined by HG-AAS ranged from 83% to 108% in liver, from 78% to 90% in lung, and from 84% to 123% in placenta. Mean concentrations of total speciated arsenic in both maternal and fetal tissues from the 85-ppm treatment group were consistently greater than in corresponding tissues from 42.5-ppm group (Table 1). However, these differences were statistically significant only for maternal lung ($p < 0.01$) and liver ($p < 0.001$). For the 42.5-ppm treatment group, mean total speciated arsenic concentrations in maternal tissues and placenta did not differ significantly. In contrast, for the 85-ppm treatment group, mean total speciated arsenic concentrations in maternal lung and liver were significantly greater than in placenta ($p < 0.001$). At either dosage level, total speciated arsenic concentrations in maternal blood and placenta did not differ significantly. In general, maternal tissues contained greater concentrations of total speciated arsenic than corresponding fetal tissues. Statistically significant differences in total speciated arsenic concentrations in corresponding maternal and fetal tissues occurred in lung in the 42.5-ppm treatment group ($p < 0.01$) and in lung and in liver in the 85-ppm treatment group ($p < 0.001$). Comparing mean total speciated arsenic concentrations in placenta with those in fetal tissues in both treatment groups found no significant differences. Mean total speciated arsenic concentrations for liver, lung, or blood did not differ significantly in male and female fetuses (data not shown).

Table 2 summarizes the percentages of total speciated arsenic present as iAs, MAs, or DMAs in maternal and fetal tissues. In general, DMAs was the major metabolite, representing from 43.6% to 88.2% of total speciated arsenic in tissues. However, iAs and MAs represented significant fractions of total speciated arsenic in maternal liver and blood, respectively. Comparing percentages of speciated arsenicals for maternal and fetal tissues from the two treatment groups found no significant differences related to arsenite dosage level. However, there were significant differences between maternal and fetal tissues in proportions of some metabolites. In both treatment groups, percentages of iAs and of DMAs in maternal and fetal liver differed significantly ($p < 0.001$). The percentage of iAs was greater and the percentage of DMAs was smaller in maternal than in fetal liver. Notably, iAs represented a greater percentage of total speciated arsenic in maternal liver than in placenta ($p < 0.01$). For both dosage levels, percentages of MAs and DMAs in maternal and fetal blood were also differed significantly ($p < 0.001$) with maternal blood containing a higher percentage of MAs and a lower percentage of DMAs than did fetal blood. The percentages of DMAs in placenta and in fetal lung, liver, or blood differed significantly ($p < 0.001$) in the 85-ppm treatment group but not in the 42.5-ppm treatment group. In the high dosage group, percentage of DMAs in all fetal tissues consistently exceeded that found in placenta.

3.2. Concentration dependence of arsenic species in maternal and fetal tissues

For all tissues, relationships between concentrations of each arsenic species and total speciated arsenic concentrations were examined (Fig. 1). Here, comparisons were made on a tissue concentration basis, not based on iAs dosage level. In maternal lung, DMAs was the predominant species over the entire range of total speciated arsenic concentrations. The relative contributions of iAs, MAs, and DMAs to tissue arsenic burden in maternal liver changed as a function of total speciated arsenic concentration. At low total speciated arsenic concentrations, most tissue arsenic was DMAs. At higher total speciated arsenic concentrations, iAs predominated. In maternal blood, roughly equal concentrations of MAs and DMAs were found at lower total speciated arsenic concentrations; at higher concentrations, DMAs was predominant. Maternal blood is the proximate source of arsenicals for transfer across placenta. Thus, it is likely that the placenta and fetal mouse were exposed mostly to MAs and DMAs. For both placenta and fetal tissues, DMAs was the predominant arsenical for all total speciated arsenic concentrations. In these tissues, DMAs concentration increased proportionally to the concentration of total speciated arsenic. Concentrations of arsenical species in placenta roughly paralleled those in maternal blood. However, concentrations of arsenical species in fetal liver, lung, and blood were consistently lower than concentrations in corresponding maternal tissues.

4. Discussion

Although considerable attention has been devoted to assessing the potential of iAs as a teratogen or reproductive toxin (DeSesso et al., 1998), less emphasis has been placed on the potential action of iAs or a metabolite as a transplacental carcinogen. However, the transplacental exposure model for iAs used in this study has been definitively linked to occurrence of cancers in several tissues (Waalkes et al., 2003,2004a). Hence, exposure *in utero* to iAs or a metabolite leads to irreversible cellular transformation and a malignant phenotype. These findings are consistent with the hypothesis that iAs or a metabolite crosses the placenta and produces effects during a period of rapid growth and development when susceptibility to the carcinogenic actions of many chemicals is heightened (Anderson et al., 2000). As is typical in rodent carcinogenesis studies in which the number of exposed animals is restricted by practical considerations, the present study used dosage levels of iAs which are substantially higher than those commonly encountered in human population exposures. It should be noted that comparing exposures in humans consuming iAs with those produced in the mouse model used in the present study is problematic. In an Argentinian population

consuming water containing about 200 ppb of iAs, maternal blood collected in late gestation contained about 11 mu;g per l (Concha et al., 1998). By comparison, blood collected from pregnant mice at the end of gestational exposure to iAs contained 25–75 times higher concentrations of As. Hence, it is reasonable to conclude that doses of iAs and its metabolites attained in the mouse model exceeded by many-fold those found in human populations. Nevertheless, in populations that use drinking water supplies containing over 1 ppm of iAs (Guha Mazumder et al., 1998), high *in utero* exposures to iAs or its metabolites could contribute to the increased risk of cancer that has been associated with chronic exposure to iAs. Because little is known about the pattern and extent of exposure of fetal mice to iAs or its metabolites, this study quantified arsenicals in maternal and fetal tissues, examined dosage dependency of arsenical accumulation in maternal and fetal tissues, and determined relative contributions of each arsenical to total arsenic burdens of maternal and fetal tissues.

Although concentrations of total speciated arsenicals in maternal lung and liver were significantly different between dosage groups, in other maternal and fetal tissues there were no significant dosage dependencies for concentrations of total speciated arsenicals. These deviations from expected patterns of dosage dependency for tissue distribution of arsenicals may reflect differences in both kinetic and dynamic factors that affect the metabolism and disposition of arsenite and its metabolites, particularly at high dosage levels used in these studies. The lack of significant dosage-dependent differences could also reflect the small sample size or high variability in the measured concentrations of arsenicals. Significant differences between concentrations of total speciated arsenicals in maternal and fetal tissues suggested that placenta limits transfer or accumulation of arsenicals. Studies of tissue distribution of arsenic in rats (Tanaka, 1976) and mice (Gerber et al., 1982; Hood et al., 1988) following iAs exposure *in utero* found higher arsenic concentrations in placenta than in fetal tissues. Although not significantly different, lower concentrations of total speciated arsenicals in fetal tissues than in placenta were also consistent with the function of the placenta as a barrier affecting the transfer of inorganic arsenic or its metabolites from mother to fetus.

For both maternal and fetal tissues, differences between dosage groups in percentages of the total speciated arsenic concentrations accounted for by iAs, MAs, or DMAs were not significant. However, there were significant differences in proportions of these arsenicals in maternal and fetal tissues. Because iAs, MAs, and DMAs are linked in a precursor, intermediate, and product relationship, a change in the relative proportion of one arsenical species must be accompanied by change in the concentration of one or both of the other arsenicals. Hence, in both dosage groups, significant differences occurred in percentages of iAs and DMAs in maternal and fetal liver. Significant changes also occurred in percentages of MAs and DMAs present in maternal and fetal blood in both dosage groups. Differences in proportions of arsenicals in maternal and fetal tissues could arise in differences in disposition, formation, or retention of these metabolites. For example, the predominance of DMAs in fetal tissues might reflect its relative abundance in maternal blood, its preferential transfer across placenta, or its preferential retention in fetal tissues.

Notably, an earlier study in fetal mice exposed transplacentally to iAs found DMAs to be the predominant arsenical present in fetuses but did not provide data on distribution of iAs and its metabolites in fetal tissues (Hood et al., 1987). Accumulation and retention of DMAs in fetal tissues could reflect an unusual affinity of some tissue constituent for DMAs. Preferential accumulation of DMAs occurs in lungs of rodents exposed to iAs or DMAs (Marafante et al., 1987; Hughes et al., 2000; Kenyon et al., 2005). Accumulation of DMAs in lung produces morphological changes and cellular toxicity (Yamanaka and Okada, 1994; Kato et al., 2000), although, critical molecular targets in tissues for iAs and its metabolites have not been identified. Accumulation of DMAs in fetal tissues could be a consequence of its formation in placenta or in fetal tissues. Although systematic data are lacking on the ontogeny of arsenic

methylation in the developing mouse, the homologous *AS3MT* gene in the mouse (Li et al., 2005) could catalyze metabolism of arsenicals in fetal tissues. AS3MT mRNA has been detected in mouse fetuses and embryos (http://cgap.nci.nih.gov/SAGE/mSEM); however, additional information on AS3MT mRNA and protein levels and on activities in fetal tissues are needed to assess the contribution of arsenic methylation capacity to the accumulation of DMAs during gestation.

The pattern of metabolites found in tissues might also reflect a concentration-dependent change in capacity for enzymatic production of methylated metabolites. In this study, the changing pattern of arsenicals in maternal liver as a function of total speciated arsenical concentration may reflect concentration-dependent changes in capacity of liver to convert iAs to methylated metabolites. Tissue concentrations of methylated arsenicals and pattern and extent of excretion of these metabolites in urine have been reported to be dose dependent in mice that received iAs^{V} or iAs^{III} (Hughes et al., 1994, 1999; Csanaky et al., 2003). Patterns of metabolite formation in cultured primary rat or human hepatocytes exposed to iAs^{III} show that formation of DMAs from MAs is inhibited at high substrate concentration (Styblo et al., 1999; Drobná et al., 2004; Easterling et al., 2002). Thus, a decreased level of DMAs in maternal liver at high levels of exposure may be linked to high concentrations of iAs and MAs which inhibit enzymatically catalyzed formation of DMAs.

Aberrant patterns of gene expression in adult mice may be a consequence of *in utero* exposure to iAs. Tumorous and non-tumorous tissue from livers of adult male mice exposed *in utero* to iAs show aberrant patterns for expression of genes involved in cell differentiation and proliferation (Liu et al., 2004). Because arsenicals are cleared from tissues with half times of a few days (Hughes et al., 2003), persistent alterations of gene expression in the tissues of mature mice may reflect changes in gene programming caused by early life exposure to iAs or a metabolite. An altered pattern of methylation of DNA induced by exposure to iAs or a metabolite *in utero* is one plausible mechanism for an effect on gene expression. For example, hypomethylation of the promoter region of the estrogen receptor α (ER-α) gene in livers of adult male mice that were exposed *in utero* to iAs has been linked to elevated ER-α mRNA levels and is consistent with a feminized pattern of gene expression in livers of these mice (Waalkes et al., 2004c). Aberrant ER-α gene expression induced by exposure *in utero* to iAs probably contributes to increased incidence of urogenital tract tumors found in female mice treated postnatally with diethylstilbestrol (Waalkes et al., 2006). Global DNA hypomethylation has also been observed in livers of adult male mice chronically exposed to iAs in drinking water (Chen et al., 2004). These finding suggest that increased demand for AdoMet to serve as a methyl group donor for methylation of high concentrations of iAs affects the ability of cells to meet the demand for AdoMet-derived methyl groups for the maintenance of the normal pattern of DNA methylation. Indeed, a competition for methyl group donation from AdoMet might be the molecular locus for a critical effect of arsenic on gene expression (Goering et al., 1999). Because altered patterns of DNA methylation contribute to development or progression of malignant tumors (Davis and Uthus, 2004), this effect could underlie malignant transformations seen in tissues of mice long after *in utero* exposure to iAs or its metabolites has ended. Although iAs and it metabolites, MAs and DMAs, were found in fetal tissues that are targets for tumor development in later life, the arsenical species responsible for transplacental carcinogenesis has not been determined. DMAs, the most abundant arsenical in fetal tissues, is a complete carcinogen and a promoter in adult rats (Yamamoto et al., 1995; Wei et al., 1999) and a promoter in adult mice (Mizoi et al., 2005). Additional studies of the disposition of DMAs and other intermediates and products in the pathway for arsenic methylation using the transplacental exposure model in the mouse may identify critical species and clarify molecular processes that underlie these adverse effects.

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References

Ahmad SA, Sayed MH, Barua S, Khan MH, Faruquee MH, Jalil A. Arsenic in drinking water and pregnancy outcome. Environ Health Perspect 2001;109:629–631. [PubMed: 11445518]

Aldick HJ, Fabry H. Multiple basilome durch arsenein-wirkung in der fetalperiode. Der Hautarzt 1973;24:496.

Anderson LM, Diwan BA, Fear NT, Roman E. Critical windows exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Perspect 2000;108(Suppl 3):573–594. [PubMed: 10852857]

Bolliger CT, van Zijl P, Louw JA. Multiple organ failure with the adult respiratory distress syndrome in homicidal arsenic poisoning. Respiration 1992;59:57–61. [PubMed: 1315982]

Chakraborti D, Mukherjee SC, Pati S, Sengupta MK, Rahman MM, Chowdhury UK, Lodh D, Chands CR, Chakraborti AK, Basu GK. Arsenic groundwater contamination in Middle Ganga Plain, Bihar India: a future danger? Environ Health Perspect 2003;111:1194–1201. [PubMed: 12842773]

Chen H, Li S, Liu J, Diwan BA, Barrett JC, Waalkes MP. Chronic inorganic arsenic exposure induces hepatic global and individual gene hypomethylation: implications for arsenic hepatocarcinogenesis. Carcinogenesis 2004;25:1779–1786. [PubMed: 15073043]

Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. Exposure to inorganic arsenic metabolites during early human development. Toxicol Sci 1998;44:185–190. [PubMed: 9742656]

Csanaky I, Nemeti B, Gregus Z. Dose-dependent biotransformation of arsenite in rats—not *S*-adenosylmethionine depletion impairs arsenic methylation at high dose. Toxicology 2003;183:77–91. [PubMed: 12504344]

Cullen WR, McBride BC, Reglinski J. The reduction of trimethylarsine oxide to trimethylarsine by thiols: a mechanistic model for the biological reduction of arsenicals. J Inorg Biochem 1984;21:45–60.

Davis CD, Uthus EO. DNA methylation, cancer susceptibility, and nutrient interactions. Exp Biol Med 2004;229:988–995.

DeSesso JM, Jacobson CF, Scialli AR, Farr CH, Holson JF. An assessment of the developmental toxicity of inorganic arsenic. Reprod Toxicol 1998;12:385–433. [PubMed: 9717692]

Devesa V, Del Razo LM, Adair B, Drobna Z, Waters SB, Hughes MF, Styblo M, Thomas DJ. Comprehensive analysis of arsenic metabolites by pH-specific hydride generation atomic absorption spectrometry. J Anal At Spectrom 2004;19:1460–1467.

Drobná Z, Waters SB, Walton FS, LeCluyse EL, Thomas DJ, Styblo M. Interindividual variation in the metabolism of arsenic in cultured primary human hepatocytes. Toxicol Appl Pharmacol 2004;201:166–177. [PubMed: 15541756]

Easterling MR, Styblo M, Evans MV, Kenyon EM. Pharmacokinetic modeling of arsenite uptake and metabolism in hepatocytes—mechanistic insights and implications for further experiments. J Pharmacokinet Pharmacodyn 2002;29:207–234. [PubMed: 12449496]

Gerber GB, Maes J, Eykens B. Transfer of antimony and arsenic to the developing organism. Arch Toxicol 1982;49:159–168. [PubMed: 7059282]

Germolec DR, Spalding J, Yu HS, Chen GS, Simeonova PP, Humble MC, Bruccoleri A, Boorman GA, Foley JF, Yoshida T, Luster MI. Arsenic enhancement of skin neoplasia by chronic stimulation of growth factors. Am J Pathol 1998;153:1775–1785. [PubMed: 9846968]

Goering PL, Aposhian HV, Mass MJ, Cebrian M, Beck BD, Waalkes MP. The enigma of arsenic carcinogenesis: role of metabolism. Toxicol Sci 1999;49:5–14. [PubMed: 10367337]

Guha Mazumder DN, Haque R, Ghosh N, De BK, Santra A, Chakraborty D, Smith AH. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal India. Int J Epidemiol 1998;27:871–877. [PubMed: 9839746]

Holson JF, Desesso JM, Jacobson CF, Farr CH. Appropriate use of animal models in the assessment of risk during prenatal development: an illustration using inorganic arsenic. Teratology 2000;62:51–71. [PubMed: 10861633]

Hood RD. Developmental effects of methylated arsenic metabolites in mice. Bull Environ Contam Toxicol 1998;61:231–238. [PubMed: 9702361]

Hood RD, Vedel-Macrander GC, Zaworotko GC, Tatum MJ, Meeks RGFM. Distribution, metabolism, and fetal uptake of pentavalent arsenic in pregnant mice following oral or intraperitoneal administration. Teratology 1987;35:19–25. [PubMed: 3563933]

Hood RD, Vedel GC, Zaworotko MJ, Tatum FM, Meeks RG. Uptake, distribution, and metabolism of trivalent arsenic in the pregnant mouse. J Toxicol Environ Health A 1988;25:423–434.

Hopenhayn-Rich C, Browning SR, Hertz-Picciotto I, Ferreccio C, Peralta C, Gibb H. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. Environ Health Perspect 1998;108:667–673. [PubMed: 10903622]

Hughes MF, Kenyon EM, Edwards BC, Mitchell CT, Thomas DJ. Strain-dependent disposition of inorganic arsenic in the mouse. Toxicology 1999;137:95–108. [PubMed: 10521146]

Hughes MF, Del Razo LM, Kenyon EM. Dose-dependent effects on the tissue distribution and metabolism of dimethylarsinic acid in the mouse after intravenous administration. Toxicology 2000;143:155–166. [PubMed: 10755702]

Hughes MF, Kenyon EM, Edwards BC, Mitchell CT, Del Razo LM, Thomas DJ. Accumulation and metabolism of arsenic in mice after repeated oral administration of arsenate. Toxicol Appl Pharmacol 2003;191:202–210. [PubMed: 13678653]

Hughes MF, Menache M, Thompson DJ. Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. Fundam Appl Toxicol 1994;22:80–89. [PubMed: 8125217]

IARC (International Agency for Research on Cancer). Monographs on the evaluation of carcinogenic risks to humans: some drinking water disinfectants and contaminants, including arsenic. 84. 2004. p. 185-270.

Kato K, Yamanaka K, Nakano M, Hasegawa A, Okada S. 72-kDa stress protein (hsp72) induced by administration of dimethylarsinic acid to mice accumulates in alveolar flat cells of lung, a target organ for arsenic carcinogenesis. Biol Pharm Bull 2000;23:1212–1215. [PubMed: 11041253]

Kenyon EM, Del Razo LM, Hughes MF. Tissue distribution and urinary excretion of inorganic arsenic and its methylated metabolites in mice following acute oral administration of arsenate. Toxicol Sci 2005;85:468–475. [PubMed: 15703264]

Li J, Waters SB, Drobna Z, Devesa V, Styblo M, Thomas DJ. Arsenic (+3 oxidation state) methyltransferase and the inorganic arsenic methylation phenotype. Toxicol Appl Pharmacol 2005;204:164–169. [PubMed: 15808521]

Lin S, Shi Q, Nix FB, Styblo M, Beck MA, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ. A novel *S*-adenosyl-L-methionine: arsenic(III) methyltransferase from rat liver cytosol. J Biol Chem 2002;277:10795–10803. [PubMed: 11790780]

Lindgren A, Vahter M, Dencker L. Autoradiographic studies on the distribution of arsenic in mice and hamsters administered 74As-arsenite or -arsenate. Acta Pharmacol Toxicol 1982;51:253–265.

Liu J, Xie Y, Ward JM, Diwan BA, Waalkes MP. Toxicogenomic analysis of aberrant gene expression in liver tumors and nontumorous livers of adult mice exposed *in utero* to inorganic arsenic. Toxicol Sci 2004;77:249–257. [PubMed: 14691202]

Lugo G, Cassady G, Palmissano P. Acute maternal arsenic intoxication with neonatal death. Am J Dis Child 1969;117:328–330. [PubMed: 5765153]

Ma M, Le XC. Effect of arsenosugar ingestion on urinary arsenic speciation. Clin Chem 1998;44:539–550. [PubMed: 9510859]

Marafante E, Vahter M, Norin H, Envall J, Sandstrom M, Christakopoulos A, Ryhage R. Biotransformation of dimethylarsinic acid in mouse, hamster and man. J Appl Toxicol 1987;7:111–117. [PubMed: 3624766]

Mizoi M, Takabayashi F, Nakano M, An Y, Sagesaka Y, Kato K, Okada S, Yamanaka K. The role of trivalent dimethylated arsenic in dimethylarsinic acid-promoted skin and lung tumorigenesis in mice: tumor-promoting action through the induction of oxidative stress. Toxicol Lett 2005;158:87–94. [PubMed: 16039397]

Rogers EH, Chernoff N, Kavlock RJ. The teratogenic potential of cacodylic acid in the rat and mouse. Drug Chem Toxicol 1981;4:49–61. [PubMed: 7261946]

Rossman TG. Mechanism of arsenic carcinogenesis: an integrated approach. Mutat Res 2003;533:37–65. [PubMed: 14643412]

Stockwell PB, Corns WT. Environmental sensors based on atomic fluorescence. Analyst 1994;119:1641–1645.

Styblo M, Del Razo LM, LeCluyse EL, Hamilton GA, Wang C, Cullen WR, Thomas DJ. Metabolism of arsenic in primary cultures of human and rat hepatocytes. Chem Res Toxicol 1999;12:560–565. [PubMed: 10409394]

Tanaka I. Arsenic metabolism: 17-studies of placental transfer of arsenic and the effects of antidotes and diet. Nippon Yakurigaku Zasshi 1976;72:673–687. [PubMed: 795757]

Thomas DJ, Styblo M, Lin S. The cellular metabolism and systemic toxicity of arsenic. Toxicol Appl Pharmacol 2001;176:127–144. [PubMed: 11601889]

Thomas DJ, Waters SB, Styblo M. Elucidating the pathway for arsenic methylation. Toxicol Appl Pharmacol 2004;198:319–326. [PubMed: 15276411]

Waalkes MP, Liu J, Ward JM, Diwan BA. Animals models for arsenic carcinogenesis: inorganic arsenic a transplacental carcinogen in mice. Toxicol Appl Pharmacol 2004a;198:377–384. [PubMed: 15276417]

Waalkes MP, Ward JM, Diwan BA. Induction of tumors in liver, lung, ovary, and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. Carcinogenesis 2004b; 125:133–141.

Waalkes MP, Liu J, Chen H, Xie Y, Achanazar WE, Zhou YS, Cheng ML, Diwan BA. Estrogen signaling in liver of male mice with hepatocellular carcinoma induced by exposure to inorganic arsenic *in utero*. J Natl Cancer Inst 2004c;96:466–474. [PubMed: 15026472]

Waalkes MP, Liu J, Ward JM, Powell DA, Diwan BA. Urogenital carcinogenesis in female CD1 mice induced by *in utero* arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. Cancer Res 2006;66:1337–1345. [PubMed: 16452187]

Waalkes MP, Ward JM, Liu J, Diwan BA. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol Appl Pharmacol 2003;186:7–17. [PubMed: 12583988]

Wei M, Wanibuchi H, Yamamoto S, Li W, Fukushima S. Urinary bladder carcinogenicity of dimethylarsinic acid in male F344 rats. Carcinogenesis 1999;20:1873–1876. [PubMed: 10469637]

Willhite CC, Ferm VH. Prenatal and developmental toxicology of arsenicals. Adv Exp Med Biol 1984;177:205–228. [PubMed: 6388261]

Yamamoto S, Konishi Y, Matsuda T, Murai T, Shibata MA, Matsui-Yuasa I, Otani S, Kuroda K, Endo G, Fukushima S. Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. Cancer Res 1995;55:1271–1276. [PubMed: 7882321]

Yamanaka K, Okada S. Induction of lung-specific DNA damage by metabolically methylated arsenics via the production of free radicals. Environ Health Perspect 1994;102(Suppl 3):37–40. [PubMed: 7843134]

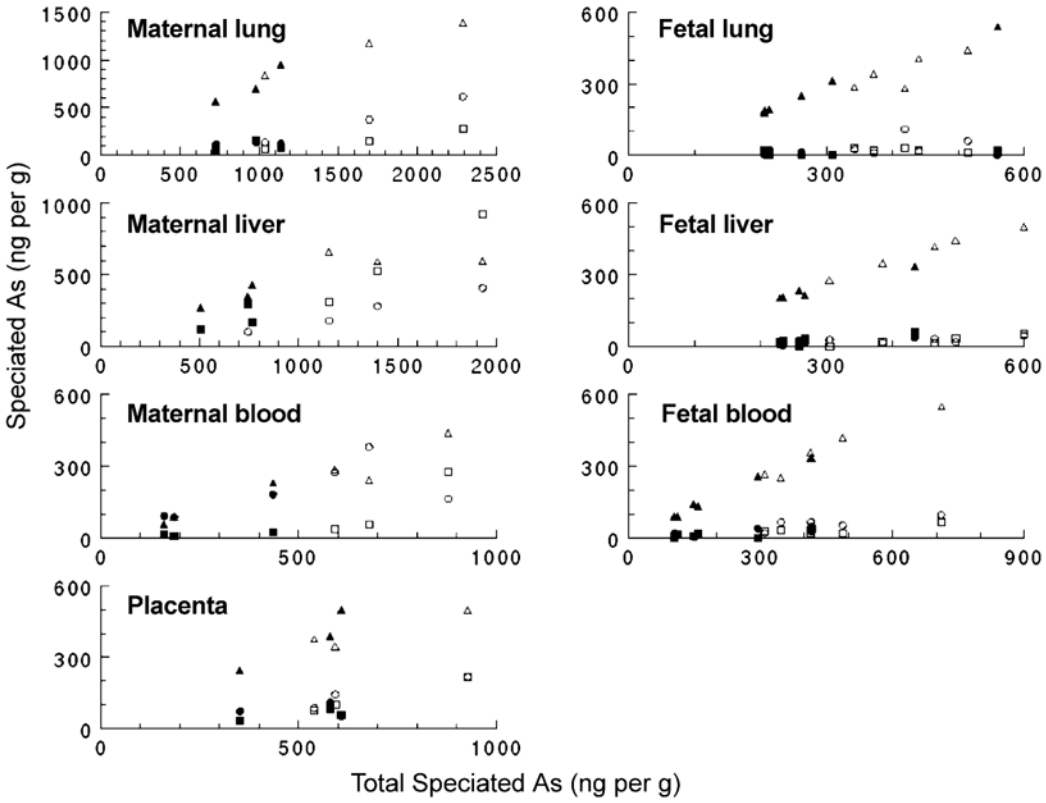

Fig. 1.

Relationship between concentrations of total speciated arsenic and concentrations of each arsenical species in maternal and fetal tissues. Data shown for inorganic arsenic (■, □), methyl arsenic (●, ○), and dimethyl arsenic (▲, △) in tissues. Filled symbols are data from maternal and fetal tissues from the 42.5 ppm arsenic dosage group; open symbols are from the 85 ppm arsenic dosage group.

Table 1

Dosage level dependence of concentrations of total speciated arsenicals in maternal and fetal mouse tissues[a]

	Concentration of inorganic As in drinking water	
	42.5 ppm	**85 ppm**
Maternal lung	949.3[a,e,g,h]	1674[a,c,f,i]
	206.7	625.4
Maternal liver		
	672.7[b,h]	
	1493.9[b,d,g,j]	
	144.1	396.7
Maternal blood	260.3[g]	716.2[i,j]
	161.7	147.1
Placenta	513.3	686.5[c,d]
	140.7	210.4
Fetal lung	292.6[e]	417.6[f]
	136.7	65.8
Fetal liver	293.2	504[g]
	88	98.2
Fetal blood	206.2	447.7
	123.6	142.8

[a–j]Values labeled with the same letter are significantly different ($p < 0.05$).

[a]Concentration of total speciated arsenic in tissues expressed as ng of arsenic per g of tissue. Mean (upper) and standard deviation (lower) shown. For each dosage level, $n = 3$; for maternal tissues and $n = 5$ or 6 for placentas and fetal tissues.

Table 2

Percentages of tissue arsenic present as inorganic (iAs), methyl (MAs), and dimethyl (DMAs) arsenicals[a]

	Concentration of inorganic As in drinking water					
	42.5 ppm			85 ppm		
	iAs	MAs	DMAs	iAs	MAs	DMAs
Maternal lung	10[b]	13.1[r]	76.9	9.1[p]	20.6	70.3
	5.5	2.4	6.2	2.9	7.2	9.9
Maternal liver	28.2[a,e,n,o]	19.5[s]	52.3[c]	37.6[b,f,p,q]	18.9[t]	43.6[d]
	9.6	5.1	4.7	10.4	2.9	13.2
Maternal blood	6.2[o]	48.2[g,r,s]	45.6[i]	15.2[q]	40.2[g,t]	44.5[j]
	1.7	7.6	9.1	14.2	19.5	7.8
Placenta	11.2[c]	16.1	72.7	18.2[f]	21.3	60.5[k,l,m]
	2.6	6.5	7.8	4.5	4.5	8.4
Fetal lung	3.5	3.2	93.2	5.8	10.1	84.0[k]
	4.4	3.9	4.7	2.7	9.6	10.3
Fetal liver	6.9[a]	6.1	88.2[c]	9[b]	7.6	83.5[d,l]
	5.2	3.2	3.9	3.5	4.1	6.8
Fetal blood	5.9	9.8[g]	84.3[i]	6.9	11.9[h]	81.4[j,m]
	5.3	5.3	6.2	2.5	5.1	5.5

[a–s]Values labeled with the same letter are significantly different ($p < 0.05$).

[a]Percentages of tissue arsenic present as inorganic, methyl, or dimethyl species. Mean (upper) and standard deviation (lower) shown. For each dosage level, $n = 3$ for maternal tissues and $n = 5$ or 6 for placentas and fetal tissues.