Supplemental Material

Farzan, et al., "*In utero* and early life arsenic exposure in relation to long-term health and disease".

Supplemental Table 1. Epidemiological studies of *in utero* exposure to arsenic and later life health and mortality outcomes.

Author	Population/ Study Group	Study Design	Sample Size	Exposure Assessment	Exposure Level	Outcomes	Mortality/Risk	Main Findings
Rosenberg, 1973	Region II, Chile (infants, age 2)	Case series	N=2	Historical As water contamination	Unknown (average: 870 µg/L)	Autopsy-Death from MI	NA	Infants had hallmarks of chronic As exposure, likely *in utero* and in infancy. Both died of MI and had vascular lesions resembling atherosclerosis.
Rosenberg, 1974	Region II, Chile (infants & children, ages 2-16)	Case series	N=5	Historical As water contamination	Unknown (average: 870 µg/L)	Autopsy-Death from MI, broncho-pneumonia, liver cirrhosis	NA	All children had similar vascular lesions, arterial thickening and had evidence of chronic As exposure, which likely occurred *in utero* or in early childhood.
Smith, et al., 2006	Region II, Chile	Ecologic	N=224 lung-related deaths	Historical As water contamination	Unknown (average: 870 µg/L)	Lung cancer, bronchiectasis and COPD mortality in 30-49 year olds, 1989-2000	Born 1950-57 Lung cancer SMR= 7.0, 95% CI: 5.4-8.9 Bronchiectasis SMR= 12.4, 95% CI: 3.3-31.7 Born 1958-70 Lung cancer SMR= 6.1, 95% CI: 3.5-9.9 Bronchiectasis SMR= 46.2, 95% CI: 21.1-87.7	Those born just before the high-exposure period (1950-1957) and exposed in early childhood had increased mortality from lung cancer and bronchiectasis. Those likely exposed in utero and in early childhood had similarly elevated lung cancer mortality for lung cancer and very high mortality rates for bronchiectasis
Yuan, et al., 2007	Region II, Chile	Ecologic	N= 79,430 deaths	Historical As water contamination	Unknown (average: 870 µg/L)	Mortality and CVD related mortality, 1950-2000	MI mortality rate ratios During peak exposure Male = 1.48, 95% CI: 1.37-1.59 Female = 1.26, 95% CI: 1.14-1.40 Post-peak exposure Males 30-49yrs, born 1958-70 = 3.23, 95% CI: 2.79-3.75	Highest risk of MI was for young adult men 30-49 yrs, who were born during the high-exposure period. MI mortality was predominant cause of excess deaths during and immediately after the high-exposure period, with increased acute risks of MI mortality during the high-exposure period.
Liaw, et al., 2008	Region II, Chile	Ecologic	N= 225 all cancers; 13 liver cancer 1950-81	Historical As water contamination	Unknown (average: 870 µg/L)	All cancer and liver cancer mortality in children, 0-19 years, 1950-2000	Child liver cancer relative risk Pooled= 10.6, 95% CI: 2.9-39.2 Male= 8.9, 95% CI: 1.7-45.8 Female= 14.1, 95% CI: 1.6-126	Common childhood cancers were not increased, but childhood liver cancer mortality occurred at much higher rates than expected.
Dauphine, et al., 2011	Region II, Chile	Ecologic	N=32	Historical As water contamination	Unknown (average: 870 µg/L)	Spirometry measures of forced expiratory volume in 1 sec (FEV_1), forced vital capacity (FVC) and survey assessment of respiratory symptoms	Arsenic-associated functional decreases FEV_1= 11.5%, p trend= 0.04 FVC= 12.2%, p trend= 0.04 Breathlessness prevalence OR= 5.94, 95% CI: 1.36-26.0	Early-life arsenic exposure was associated with lower FEV_1, lower FVC, and increased breathlessness in adulthood. Dose-dependent relationships between early-life arsenic exposure and adult FEV_1 and FVC were also identified.

Smith, et al., 2012	Region II, Chile	Ecologic	N= 128 all cancer, 9 bladder cancer, 1 laryngeal cancer, 9 liver cancer, 12 renal disease in those born 1958-1970	Historical As water contamination	Unknown (average: 870 µg/L)	Mortality in young adults 30-49 years, born between 1958-1970	<u>Bladder cancer SMR</u> Pooled= 18.1, 95% CI: 11.3-27.4 Males born 1958-70= 65.7, 95% CI: 24.1-143 Females born 1958-70= 43.0, 95% CI: 8.9-126 <u>Laryngeal cancer SMR</u> Pooled= 8.1, 95% CI: 3.5-16.0 <u>Liver cancer SMR</u> Pooled= 2.5, 95% CI: 1.6-3.7 <u>Chronic renal disease SMR</u> Pooled= 2.0, 95% CI: 1.5-2.8	Increased mortality from bladder cancer, laryngeal cancer, liver cancer, and chronic renal disease was observed for in individuals exposed in utero or as children (<18yrs). The greatest increase in mortality was observed for bladder cancer in men and women likely exposed *in utero*.
Hawkesworth, et al 2012	Matlab, Bangladesh MINIMat study	Prospecti ve cohort (birth - 4.5 years)	N= 1887	Maternal U-As (GW 8)	Median: 80µg/L	SBP and DBP in children at 18 months and 4.5 years of age; kidney volume; GFR	1 mg/L increase in maternal U-As was associated with increase in: Child SBP: 3.69 mmHg 95% CI: 0.74, 6.63; p= 0.01 Child DBP: 2.91 mmHg 95% CI: 0.41, 5.42; p= 0.02 1 mg/L increase in child U-As was associated with increase in: Child SBP: 8.25 mmHg 95% CI: 1.37, 15.1; p= 0.02 1 mg/L increase in child U-As was associated with decrease in: GFR: -33.4 ml/min/1.72 m^2 95% CI: -70.2, 3.34; p= 0.08	Increased maternal urinary As was associated with increased SBP and DBP at 4.5 years of age. Increase in DBP at 4.5 years was associated with a child's U-As at 18 months. A marginal inverse association was found between child's U-As at 18 months and GFR at 4.5 years. Cadmium was also assessed and no associations were found. Although effect sizes were modest, arsenic exposure in early life could have long-term consequences for blood pressure and kidney function.
				Maternal U-As (GW 30)	Median: 83µg/L			
				Child U-As (18 mo)	Median: 34µg/L			
				Maternal urine As, GW 30	Median: 85 µg/L 5-95 percentiles: 20-508			
				Maternal blood As, GW 14	Median: 4.7 µg/kg 5-95 percentiles: 1.4- 22.2			

Abbreviations: U-As: urinary arsenic, MI: myocardial infarction, CVD: cardiovascular disease, SBP: systolic blood pressure, DBP: diastolic blood pressure, GFR: glomerular filtration rate

Supplemental Table 2. Animal models of in utero arsenic exposure and adverse health outcomes.					
Author	**Purpose/ Hypothesis**	**Model system**	**Route of exposure and arsenic dose**	**Arsenic Exposure Assessments**	**Findings**
Waalkes, et al., 2003	Examine ability of arsenic to induce cancer transplacentally	Pregnant C3H mice	0, 42.5, 85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth	Necropsy, tumor incidence	Male offspring exposed in utero to arsenic had increased dose-dependent incidence of hepatocellular carcinoma, adrenal tumor incidence and multiplicity and increases in liver tumor multiplicity. In female offspring, increases occurred in ovarian tumors, lung carcinomas and proliferative lesions of the uterus and oviduct.
Waalkes, et al., 2004	Examine ability of transplacental arsenic exposure to promote TPA-induced carcinogenesis	Pregnant C3H mice	0, 42.5, 85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth; Topical 12-O-tetradecanoyl phorbol-13-acetate (TPA) 2x weekly from 4-25 weeks of age	Necropsy, tumor incidence	Arsenic-induced dose-dependent increases in hepatocellular carcinoma incidence and multiplicity and increases in adrenal tumor incidence and multiplicity were observed regardless of TPA exposure in male offspring Female offspring had increased epithelial ovarian tumors and pre-neoplastic lesions of the reproductive tract, regardless of TPA exposure. TPA had no effect on skin tumors, but promoted arsenic related liver tumors in females and lung tumors in both males and females.
Waalkes, et al., 2004	Examine ability of transplacental arsenic exposure to promote TPA-induced carcinogenesis	Pregnant C3H mice	0, 42.5, 85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth; Topical TPA after birth (2 μg/0.1 ml acetone, twice/week for 21 weeks)	Necropsy, tumor incidence	Male offspring developed hepatocellular carcinoma and adrenal tumors after in utero exposure. Female offspring developed lung carcinoma, ovarian tumors, and uterine and oviduct pre-neoplasia. Combined arsenic and TPA treatment induced a significant increase in hepatocellular tumors in female offspring, but arsenic alone was not effective. In utero arsenic exposure can act as a complete carcinogen but can also act as a co-carcinogen in the female liver.
Waalkes, et al., 2006	Examine ability of transplacental arsenic exposure to promote DES or tamoxifen induced carcinogenesis in male offspring	Pregnant CD1 mice	85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to 18	Necropsy, tumor incidence	In male CD1 mice, in utero arsenic exposure alone induced liver adenoma and carcinoma, lung adenocarcinoma, adrenal adenoma and renal cystic hyperplasia. Additional treatment with postnatal DES enhanced arsenic-induced hepatocarcinogenesis. In utero arsenic initiated urinary bladder tumors when followed with postnatal tamoxifen and uroepithelial proliferative lesions when followed with tamoxifen or DES.
Waalkes, et al., 2006	Examine ability of transplacental arsenic exposure to promote DES or tamoxifen induced carcinogenesis in female offspring	Pregnant CD1 mice	85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to 18	Necropsy, tumor incidence	In female CD1 mice, in utero arsenic alone induced some urogenital system tumors, including mostly benign tumors of the ovary and uterus, and adrenal adenoma. DES alone induced some tumors, but when given after in utero arsenic, arsenic and DES acted synergistically to greatly enhance urogenital tumor incidence, multiplicity, and progression. Tamoxifen increased arsenic-induced uroepithelial proliferative lesions.
Liu, et al., 2007	Examine ability of transplacental arsenic exposure to promote TPA-induced carcinogenesis	Pregnant C3H mice	85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth; Topical TPA after birth (2 μg/0.1 ml acetone, twice/week for 21 weeks)	Necropsy, tumor incidence, gene and protein expression by microarray, RT-PCR and Western blot analysis	Comparison of liver tumors and normal liver samples taken from adult male and female mice showed that arsenic/TPA treatment increased expression of α-fetoprotein, k-ras, c-myc, estrogen receptor-α, cyclin D1, cdk2na, plasminogen activator inhibitor-1, cytokeratin-8, cytokeratin-18, glutathione S-transferases and insulin-like growth factor binding proteins in liver and liver tumors from both male and female mice. Arsenic/TPA decreased the expression of BRCA1, betaine-homocysteine methyltransferase, CYP7B1, CYP2F2 and insulin-like growth factor-1 in liver tumors and normal livers.
Xie, et al., 2007	Define the early molecular changes in the liver associated with transplacental arsenic exposure	Pregnant C3H mice	85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to 18	Microarray and RT-PCR	57 ng/g of arsenic was found in newborn liver, indicating arsenic had crossed the placenta and reached the fetal liver. Global methylation of hepatic DNA was not altered by arsenic, but a significant reduction in methylation occurred globally in GC-rich regions. Arsenic exposure increased expression of genes related to glutathione production, cdk-inhibitors and metallothionein-1. Arsenic deregulated expression of genes related to insulin growth factor signaling pathways and cytochrome P450 enzymes and decreased expression of betaine-homocysteine methyltransferase and thioether S-methyltransferase.
Shen, et al., 2007	Define the early molecular changes	Pregnant C3H	85 ppm $NaAsO_2$ in dams' drinking water from	PCR of genes of interest from fetal lung	Transplacental arsenic exposure increased ER-α mRNA and protein levels in the fetal lung, as well as overexpression of insulin growth factor, estrogen-

	in the lung associated with transplacental arsenic exposure	mice	gestational day 8 to 18	tissue, western blot and tumor immunohistochemical staining of ER-alpha	regulated genes (trefoil factor-3, anterior gradient-2) and steroid metabolism genes (17-β-hydroxysteroid dehydrogenase type 5 and aromatase). Lung cancer associated proteins α-fetoprotein, epidermal growth factor receptor, L-myc, and metallothionein-1 were all overexpressed in the fetal lung after in utero arsenic exposure. Lung adenoma and adenocarcinoma from adult female mice exposed to arsenic in utero showed widespread, intense nuclear ER-alpha expression.
Srivastava, et al., 2007	Examine the effect of *in utero* As exposure on the development of atherosclerotic disease	Pregnant ApoE knockout (ApoE-/-) mice	85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth	Blood lipids, aortic lesion development, vasoresponsiveness	Mice exposed to As *in utero* showed a >2-fold increase in lesion formation in the aorta, as compared to controls at both 10 and 16 weeks of age. As exposed mice also had a 20-40% decrease in total triglycerides, but no change in total cholesterol, phospholipids and total abundance of VLDL or HDL particles, but subfractionation of VLDL particles showed a decrease in large VLDL particles. As-exposed mice showed a vasorelaxation defect in response to acetylcholine indicating a defect in endothelial cell signaling. These results indicate that *in utero* As exposure induces an early onset of atherosclerosis in ApoE(-/-) mice, even in the absence of a high fat diet.
Waalkes, et al., 2008	Examine the potential for *in utero* As exposure to exacerbate skin carcinogenesis in a sensitive model	Pregnant Tg.AC mice	0, 42.5, 85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth; Topical TPA after birth (2 μg/0.1 ml acetone, twice/week for up to 40 weeks)	Tumor incidence, immunohistochemistry, RT-PCR	In utero arsenic treatment before TPA increased SCC aggression and SCC multiplicity (3-fold more than TPA alone). Arsenic plus TPA increased tumor v-Ha-ras, as well as CD34, a marker for both KSCs and skin cancer stem cells, and Rac1, a stimulator of KSC self-renewal, indicating an increased population of likely cancer stem cells, Arsenic-treated fetal skin also had increased v-Ha-ras, CD34 and Rac1.
Petrick, et al., 2009	Characterize the effects of in utero arsenic exposure on the developing lung	Pregnant Sprague-Dawley rats	500 ppb $NaAsO_2$ in dams' drinking water from gestational day 8 to 18	RT-PCR and western blotting of fetal lung tissues, pathway analysis	In utero arsenic exposure altered the expression of 59 genes and 34 proteins in the fetal lung, including those involved in B-catenin (Wnt) signaling, extracellular matrix maintenance, and fetal lung development regulator, sprouty-2.
Lantz, et al., 2009	Characterize the effects of low level in utero arsenic exposure on the developing lung	Pregnant C57BL6 mice	5, 10, 50, 100 ppb $NaAsO_2$ in dams' drinking water from gestational day 8 to 18	Immunohistochemistry, lung morphometry, RT-PCR and western blotting of fetal lung tissues; lung function	Arsenic exposure increased airway reactivity in pups in a non-reversible manner. Arsenic increased smooth muscle actin in the lung in a dose dependent manner, especially around airways smaller than 100 μm in diameter. Arsenic exposure also caused alterations in extracellular matrix protein expression.
Tokar, et al., 2010	Examine ability of transplacental arsenic exposure to promote TPA-induced carcinogenesis in different mouse strain	Pregnant Tg.AC mice	0, 42.5, 85 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth; Topical TPA after birth (2 μg/0.1 ml acetone, twice/week for 21 weeks)	Necropsy, tumor incidence	Arsenic increased adrenal cortical adenomas, independent of TPA. Arsenic increased urinary bladder hyperplasia in males, but only with TPA. Arsenic-treated females had UB hyperplasia and papillomas and had uterine hyperplasia and tumors independent of TPA.
Tokar, et al., 2011	Examine the effects of "whole-life" arsenic exposure	CD1 mice	0, 6, 12, 24 ppm $NaAsO_2$ in the drinking water 2 weeks prior to breeding, during pregnancy, lactation, and after weaning through adulthood	Necropsy, tumor incidence	In both sexes, arsenic increased incidence of adrenal tumors, lung adenocarcinoma and hepatocellular carcinoma. Gallbladder tumors were increased in males and in females uterine carcinomas and ovarian tumors were increased with arsenic dose. Arsenic-induced tumors were found at very similar target sites, although tumors from whole-life exposure were generally more aggressive and frequent.
States, et al., 2012	Test whether *in utero* As exposure is impacting the development of atherosclerotic disease via the altered hepatic development	Pregnant ApoE knockout (ApoE-/-) mice	49 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to birth	Microarray analysis of hepatic transcriptome (mRNA and microRNA) at postnatal days 1 and 70	Mice exposed to As *in utero* had altered mRNA and microRNA profiles and a 51-gene signature was identified. Genes upregulated included HSP70, a stress related pro-inflammatory protein that likely promotes atherogenesis, as well as genes involved in antigen processing and presentation, complement and coagulation, and protein export. Genes downregulated were involved in glycolysis and gluconeogenesis. SREBP was also elevated and of the altered genes, ~16% had SREBP binding sites, which likely has important impacts on cholesterol and insulin regulation, as well as adipogenesis and triglyceride synthesis.

Ngalame, et al., 2012	Determine how in utero arsenic changes the expression of Hsp70 and Hsc70 during early postnatal development	Pregnant ApoE knockout (ApoE-/-) mice	49 ppm $NaAsO_2$ in dams' drinking water from gestational day 8 to 18	Global and CpG methylation analysis and western blot analysis of livers from GD18 fetal mice and 3, 10, and 24 week old mice	Hsp70 induction was observed at 3 and 10 weeks, but not at GD18 or 24 weeks. Arsenic did not affect global DNA methylation or promoter region methylation, but methylation was increased within the Hsp70 transcribed region. Delayed induction of Hsp70 may cause a transient state of stress, predisposing to later life disease.
Kozul-Horvath, et al., 2012	Test effect of low-dose *in utero* exposure to As on mouse fetal and postnatal development	Pregnant C57B6/J mice	10 ppb $NaAsO_2$ in dams' drinking water from gestational day 1 to birth and/or up to postnatal day 30	Birth outcomes (birth weight, gestational length, litter size), postnatal growth, breast milk nutrition and triglyceride levels in dams	Arsenic exposure *in utero* and postnatally resulted in decreased growth in the pups, primarily due to decreased nutrient content in the dams' milk, as cross-fostering experiments were able to reverse the effect. The effect appeared to be more pronounced in female pups and took longer to resolve (up to six weeks) after cessation. Dams exposed to arsenic showed increased liver triglyceride levels, as well as decreased serum and milk triglycerides.
Ramsey, et al., 2013	Investigate role of early life arsenic exposure in lung development and innate immunity	Pregnant BALB/c, C57BL/6, and C3H/HeARC mice	100 ppb $NaAsO_2$ in dams' drinking water from gestational day 1 to birth	Lung growth and mechanics, microarray analysis	C57BL/6 mice were the most susceptible to effects of arsenic, with overall smaller size, smaller lungs, and impaired lung mechanics compared with controls. In utero arsenic exposure up-regulated expression of mucus production genes (Clca3, Muc5b, Scgb3a1), innate immunity genes (Reg3γ, Tff2, Dynlrb2, Lplunc1), and lung morphogenesis (Sox2). Arsenic also induced mucous cell metaplasia and increased expression of CLCA3 protein in the large airways.
Ramsey, et al., 2013	Investigate role of low dose early life arsenic exposure in lung development and mechanics	Pregnant C57BL6 mice	0, 10, 100 ppb $NaAsO_2$ in dams' drinking water from gestational day 1 to birth	Lung volume, lung mechanics, and pressure-volume curves in offspring at 2, 4, 6 and 8 weeks of age.	In utero arsenic was associated with low birth weight and impaired parenchymal lung mechanics in early life, to which male offspring were more susceptible. However, lung function in both males and females appeared to be recovered by adulthood.