

Arsenic

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1.1 Chemistry, Production, and Use

Arsenic is a naturally occurring element widely distributed in the earth’s crust and is classified chemically as a metalloid, having both properties of a metal and a non-metal. Arsenic can be found in rock, soil, water, air and the earth biosphere. There are many forms, or species, of arsenic and these can be broadly categorized as inorganic or organic (ATSDR 2007; California EPA 2004; EFSA 2009; Francesconi and Kuehnelt 2004; IPCS 2001). Under moderately reducing conditions, arsenite (+3) may be the dominant form. Under normal environmental conditions, the most stable forms, and thus most readily detected forms, are in oxidation state +5, e.g., arsenate, dimethylarsinate, arsenobetaine, arsenosugars (EFSA 2009; IPCS 2001).

The main inorganic forms of arsenic relevant for human exposures are pentavalent arsenic (also called arsenate, As(V), or As⁺⁵) and trivalent arsenic (also called arsenite, As(III), or As⁺³). These inorganic species undergoes a series of reduction and oxidative/methylation steps in human liver and other tissues to form tri- and pentavalent methylated metabolites of methylarsonite [MA(III)], methylarsonate [MA(V)], dimethylarsinite [DMA(III)], and dimethylarsinate [DMA(V)]. Some mammalian species also produce trimethylated metabolites, trimethylarsine oxide

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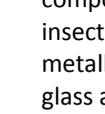
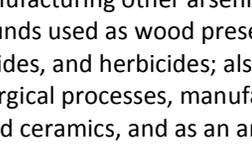
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[TMA(V)O] and, possibly, the volatile trimethylarsine [TMAs(III)] . Fish and other seafood are the major sources of exposure to organic arsenic, in the form of arsenobetaine, arsenosugars, and arsenolipids. The distinction between inorganic and organic forms is important because it is generally accepted that the organic species are excreted more quickly from the body and generally considered less toxic, with a relative rank order of As(III) > As(V) >> MA(V), DMA(V) >> arsenobetaine. However, the methylated trivalent metabolites, MA(III) and DMA(III), are significantly more toxic than their pentavalent counterpart and either As(III) or As(V) . In many cases, biomonitoring or environmental occurrence data are reported as total arsenic and do not distinguish between the different species. In those situations, understanding the relevant sources of arsenic is essential to evaluate potential arsenic related health effects, especially those related to inorganic arsenic exposure. [Table 1](#) describes common forms of arsenic, including those measured in NHANES or used in studies with experimental animals or *in vitro* model systems relevant to the diabetes and obesity literature.

Arsenic is mainly obtained as a byproduct of the smelting of copper, lead, cobalt, and gold ores. Arsenic has not been produced in the US since 1985 and all of the arsenic used in the US is imported although the US has consistently been the largest consumer of arsenic. As of 2003, the largest producer was China, followed by Chile and Peru (ATSDR 2007). About 90% of the arsenic produced globally is used to make arsenic trioxide (As(III) oxide, As_2O_3), an inorganic form used in the production of the wood preservative copper chromated arsenate (CCA) which is used to make “pressure-treated” lumber (ATSDR 2007). In 2003, US manufacturers of wood preservatives began a voluntary phase-out of CCA that was completed by December 31, 2003. However, wood treated prior to this date can still be used and was unaffected by the phase-out. Although CCA is no longer used in the U.S. for residential uses, CCA-treated wood products continue to be used in industrial applications. Arsenic trioxide and arsenic acid also have uses in the glassworks industry as decolorizing and fining agents to make bottle glass and other glass products (ATSDR 2007). Arsenic can also be used in lead-acid automobile batteries, as an alloying element in ammunition and solders, and as an anti-friction additive to metals for bearings. Gallium arsenides are used in semiconductors for telecommunications, light-emitting diodes, and space research, and light-emitting diodes. Inorganic arsenic compounds were previously used as pesticides although use for this purpose began to cease in the 1960s. Certain organic arsenic compounds continue to be used as pesticides, mostly for cotton fields and orchards [cacodylic acid (DMA(V), and two salts of MA(V): disodium methyl arsenate or DSMA, and monosodium methylarsenate or MSMA]. Some organic arsenic compounds are used as antimicrobial additives in animal feed and plastic materials (ATSDR 2007; California EPA 2004). Organic arsenic in the environment, such as roxarsone in chicken litter that is then applied as a fertilizer, is converted to inorganic arsenic after spreading. Arsenic trioxide (As_2O_3) has been used as a therapeutic agent for treatment of specific forms of leukemia (ATSDR 2007)

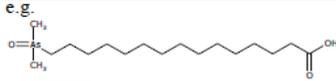
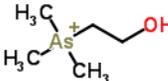
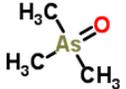
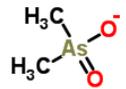
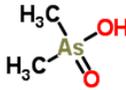
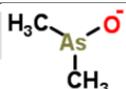
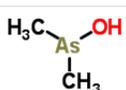
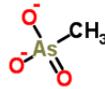
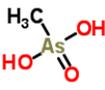
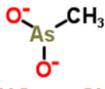
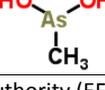
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Table 1. Common forms of arsenic					
Common Name (Systematic Name)	CAS No.	Abbreviation & Synonyms	Formula	Structure	Description
Arsenic	7440-38-2	As	As		
Arsenate	12523-21-6; 7778-43-0 (sodium salt)	As(V)	AsO ₄ ³⁻ (in basic conditions)		trace to low levels in most foods; a major form in water; considered highly toxic and carcinogenic
Arsenic acid	7778-39-4		H ₃ AsO ₄ (in acidic conditions)		
Arsenite	7784-46-5 (sodium salt)	As(III)	AsO ₃ ³⁻ (in basic conditions)		trace to low levels in most foods; considered highly toxic and carcinogenic
Arsenous acid	13464-58-9		H ₃ AsO ₃ (in acidic conditions)		
Arsenic pentoxide	1303-28-2	As(V) oxide, As pentoxide	As ₂ O ₅		commercial compound of arsenic; used as a solid or solution in the manufacturing of arsenates, weed killer, metal adhesives, insecticides, fungicides, wood preservatives, and colored gases and in printing and dyeing
Arsenic trioxide	1327-53-3	As(III) oxide, As trioxide, white As, arsenolite	As ₂ O ₃		commercial compound of arsenic used in the manufacturing other arsenic compounds used as wood preservatives, insecticides, and herbicides; also used in metallurgical processes, manufacturing of glass and ceramics, and as an anticancer drug; can be found in nature but is more commonly associated with smelting
Arsenobetaine	64436-13-1	AB or AsB, "fish arsenic"	C ₅ H ₁₁ AsO ₂ ⁻		major organic arsenic species in most seafoods; generally considered non-toxic
Arsenosugars ^(a)					major (edible algae) or significant (molluscs) arsenic species in many seafoods; mostly metabolized to DMA in humans

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Table 1. Common forms of arsenic					
Common Name (Systematic Name)	CAS No.	Abbreviation & Synonyms	Formula	Structure	Description
Arsenolipids ^(b)				e.g. 	newly discovered arsenic species present in fish oils and fatty fish; likely to be present in other seafoods as well; mostly metabolized to DMA
Arsenocholine	39895-81-3	AC or AsC	C ₅ H ₁₄ AsO		trace organic arsenic species found in seafood; readily oxidized to arsenobetaine in biological systems
Trimethylarsine oxide	4964-14-1	TMAO	C ₃ H ₉ AsO		minor organic arsenic species found in seafood; major product of As metabolism in some bacterial and animal species
Dimethylarsinate		DMA	C ₂ H ₆ AsO ₂ ⁻		minor arsenic species in seafoods and some terrestrial foods; the major human urine metabolite of iAs, arsenosugars, and arsenolipids
Dimethylarsinic acid	75-60-5		C ₂ H ₇ AsO ₂		
Dimethylarsinite		DMA (III)	C ₂ H ₆ AsO ⁻		not detected in foods; detected in some human urine samples as a metabolite of iAs; a very unstable (reactive) species that is very difficult to measure; highly toxic species considered by some researchers to be central to arsenic's mode of toxic action
Dimethylarsinous acid	55094-22-9		C ₂ H ₇ AsO		
Methylarsonate	51952-65-9	MA, MMA, monomethylarsonate	CH ₃ AsO ₃ ²⁻		trace arsenic species of some seafoods and terrestrial foods; a significant human urine metabolite of iAs
Methylarsonic acid, monomethylarsonic acid	124-58-3		CH ₅ AsO ₃		
Methylarsonite		MA (III), MMA (III), monomethylarsonite	CH ₃ AsO ₂ ²⁻		not usually detected in foods; detected in some human urine samples as a metabolite of iAs; a toxic species thought to be important for arsenic's mode of toxic action
Methylarsonous acid, monomethylarsonous acid	25400-23-1		CH ₅ AsO ₂		

From International Programme on Chemical Safety (IPCS) (2001), ATSCR (2007), Caldwell et al.(2009), European Food Safety Authority (EFSA) (2009), ChemSpider(2010)

^(a) Over 20 arsenosugars have been reported as natural products; they differ by having different R groups on the aglycone portion of the molecule, and by replacing the oxygen on the arsenic atom with either a sulfur atom or a third methyl group (see Francesconi and Edmonds (1997)). Most of the arsenic present as arsenosugars, however, is contained in just four compounds based on the structure drawn above and with (i) R=CH₂CHOHCH₂OH (EFSA 2009).

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Table 1. Common forms of arsenic

Common Name (Systematic Name)	CAS No.	Abbreviation & Synonyms	Formula	Structure	Description
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^(b) Nine arsenolipids have been reported so far as natural products, all of which contain the dimethylarsinoyl group $[(CH_3)_2As(O)-]$ bound to either one of several long chain fatty acids, or to long chain hydrocarbons. Many more arsenolipids are present in foods – their structures are currently unknown (EFSA 2009).

DRAFT

1.2 Exposure and Risk Characterizations

People can be exposed to arsenic from food and water as well as inhalation, such as breathing sawdust or smoke from burning arsenic treated wood or fly ash from combustion of As-rich coal (ATSDR 2007). Non-dietary exposure to arsenic is considered to be minor for the general population (EFSA 2009). Higher exposures may occur in people who live in areas with high natural levels of arsenic in rocks and soils or who work in jobs that involve arsenic by-products, production or use, such as copper or lead smelting, wood preservation, or pesticide manufacture or application, semiconductor manufacturing, taxidermy, or glass production. Low levels of arsenic can be found in tobacco smoke (California EPA 2004).

As(III) and As(V) are rapidly and nearly completely absorbed after ingestion and absorption of different organic arsenic compounds is generally greater than 70%. After being absorbed, arsenic is widely distributed to almost all organs and readily crosses the placental barrier. Biotransformation of inorganic arsenic in mammals includes reduction of pentavalent arsenic to trivalent arsenic species and oxidative methylation of trivalent arsenic species. Although As(III) is often described as being more toxic than As(V), the 2007 ATSDR toxicological profile for arsenic did not highlight these distinctions because: (1) the differences in relative potency were considered small, about 2-3 fold, and within the bounds of uncertainty for “no observed adverse effect levels,” or NOAELs, and “lowest observed adverse effect levels,” or LOAELs; (2) different forms of arsenic interconvert in the environment and in the body; (3) and the exact chemical speciation for human exposure, especially from water or soil, is rarely known.

Inorganic arsenic has been recognized as a human carcinogen since 1980, when it was listed as a “known” carcinogen in the 1st Report on Carcinogens and categorized as a Group 1 carcinogen by IARC (2005). Inorganic arsenic is also recognized as a carcinogen under California’s Proposition 65 (February 27, 1987) (California EPA) and the US EPA (Group A, carcinogenic to humans, IRIS, 1995) (US EPA). Cancer tissue sites include skin, lung, digestive tract, liver, bladder, kidney, and lymphatic and hematopoietic systems [reviewed in 11th Report on Carcinogens (2005)]. The most recent comprehensive assessment of arsenic conducted by a US federal agency was published by the ATSDR in August 2007 (2007). The US EPA is currently updating its Integrated Risk Information System (IRIS) assessments of inorganic arsenic. External peer review of the final assessment of cancer effects and agency review of the draft for non-cancer effects are scheduled for the 1st quarter of 2011 (status report for IRISTRACK, <http://cfpub.epa.gov/ncea/iristrac/index.cfm>). The current US EPA reference dose level is 0.3 µg/kg bw/day.

On October 22, 2009, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) released a scientific opinion on arsenic in food. The COMTAM panel was charged to assess whether the provisional tolerable weekly intake (PTWI) of 15 µg/kg bw/week (~2 µg/kg-d) established in 1988 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for inorganic arsenic was still appropriate. The CONTAM Panel concluded that the PTWI of 15 µg/kg bw/week was no longer appropriate based on data indicating that inorganic arsenic causes cancer of the lung and urinary bladder in addition to skin, and that a

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range of adverse effects had been reported at exposures lower than those reviewed in 1988. The CONTAM Panel modeled the dose-response data from key epidemiological studies and selected a benchmark response of 1% extra risk (BMDL01). The lower confidence limits of the benchmark dose for arsenic were 0.3 to 8 µg/kg-d for cancers of the lung, skin and bladder, as well as skin lesions. The estimated dietary exposures to inorganic arsenic for average and high level consumers in Europe were within the range of the BMDL01 values identified (Table 2), and led the CONTAM Panel to conclude there is little or no margin of exposure and the possibility of a risk to some consumers cannot be excluded.

The main source of arsenic in drinking water is leaching from rocks that contain arsenic. For example, from the sedimentary deposits from volcanic rocks that occurs in the basin and range provinces of Western mountain regions states of New

Mexico, Utah, Arizona, and Nevada (Frost *et al.* 2003). The “rocks” may also be tailings from old mines. Other areas within the US recognized as having higher drinking water levels of arsenic include parts of Michigan, northeastern Wisconsin, and the northeastern states (Massachusetts to Maine) due to sulfide mineral deposits in sedimentary rocks or bedrock (Frost *et al.* 2003). In Canada, arsenic “hot spots” (>10 µg/L in drinking water) have been reported in parts of Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland and Labrador, Nova Scotia, Québec, and Saskatchewan (McGuigan *et al.* 2010). Worldwide, elevated concentrations of arsenic in groundwater or drinking water are reported in Bangladesh, Taiwan (China), West Bengal (India), Peoples Republic of China (Xinjiang and Inner Mongolia), regions of Chile, North Mexico, Argentina, southwestern Finland, and Vietnam (IPCS 2001; Smedley and Kinniburgh 2002)¹. Chronic exposure to elevated levels of arsenic in drinking water is a serious public health issue, especially in places like Bangladesh where ~10 million hand-pumped wells were installed beginning in the 1970s to provide pathogen-free drinking water and prevent waterborne disease (Argos *et al.* 2010). Recent findings from a prospective cohort study, the Health Effects of Arsenic Longitudinal Study (HEALS), estimated that 21.4% of all deaths and 23.5% of deaths associated with chronic disease in this population could be attributed to arsenic exposure >10 µg/L in drinking water.

Table 2. Estimated Daily Inorganic Arsenic Intake

Region	Estimated Daily Intake (µg/kg-d)		References
	Total	Inorganic	
U.S.			
Children 2 years of age	1.8	0.31	(Tao and Bolger 1998)
Females 25-30 years of age	0.44	0.08	
Males 25-30 years of age	0.72	0.13	
Europe			
Average consumer	0.13 to 0.56		(EFSA 2009)
95th percentile consumer	0.37 to 1.22		
Children under 3 years of age ¹	0.50 to 2.66		
High consumers of algae-based products	4.0		

¹ These estimates do not include children who drink rice-drinks as a substitute for formula or cows' milk.

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On January 22, 2001 the EPA adopted a new standard, or maximum contaminant level (MCL), for arsenic in drinking water at 10 µg/L (Table 3), replacing the previous standard of 50 ppb. The drinking water standard for arsenic in drinking water is based on total arsenic including both organic and inorganic forms (US EPA), although most arsenic in drinking water is in inorganic forms. The US standard is the same as the provisional guideline for arsenic established by the WHO in 1993 at the practical quantification limit of 10µg/L, which is lower than the 1984 guideline value of 50 µg/L and is a 20-fold reduction from a maximum allowable concentration of 200 µg/L set in 1958. In 2004, the California Environmental Protection Agency established a long-term objective (called a public health goal, or PHG) for a maximum drinking water concentration for arsenic of four parts per trillion, 2500 times lower than the EPA's MCL of 10 ppb. Four parts per trillion is a level of arsenic in drinking water that would not be expected to pose a significant human health risk (California EPA 2004).

Based on an EPA study of tap water, drinking water in the US generally contains an average of ~2.4 µg/L of arsenic [EPA

1982c as cited in ATSDR (2007)]. A 1997 report from the National Arsenic Occurrence Survey reported that the percentages of water systems that would be out of compliance for arsenic MCLs of 20, 10, 5, and 2 µg/L are estimated to be 1.7, 3.6, 9.3, and 20.7%, respectively [Frey and Edwards (1997) as reviewed by (ATSDR 2007)], although 12% of water supplies from surface water sources in the North Central region of the country and 12% of supplies from groundwater sources in the Western region have levels exceeding 20 µg/L [Karagas et al., 1998 as cited in ATSDR (2007)].

Frost et al. (2003) used data from the US EPA Arsenic Occurrence and Exposure Database and additional data from state health and environment departments and water utilities to identify counties in the US that had arsenic drinking water concentrations of ≥ 10 µg/L. Thirty three counties from 11 states were identified.² When water quality data for all counties with arsenic

Table 3. Arsenic Drinking Water Standards

Country	Water Concentration (µg/L, ppb)	References
U.S. Standards		
US EPA	10 MCL/0 MCLG	US EPA , 2006) ¹¹
California EPA	0.004 PHG for cancer 0.9 PHG for non-cancer	California EPA (2004)
International Standards		
WHO	10 (guideline)	WHO (2008)
Canada	10 MAC	Health Canada (2006)
Mexico	35	Meza (2004)

Maximum contaminant level (MCL): The highest level of a contaminant that is allowed in drinking water (enforceable). MCLs are set as close to MCLGs as feasible using the best available treatment technology and taking cost into consideration.

Maximum Contaminant Level Goal (MCLG): The level of a contaminant in drinking water below which there is no known or expected risk to health (non-enforceable public health goal).

Maximum Acceptable Concentration (MAC)

Public Health Goal (PHG): A level of a contaminant in drinking water that does not pose a significant short-term or long-term health risk. A PHG is not a regulatory requirement, but a goal that California's public water suppliers and regulators should strive to meet if it is feasible to do so.

² State, counties, and estimated mean arsenic concentrations: Arizona: La Paz (11.7 µg/L), Pinal (10.3 µg/L); California: Kings (16.1 µg/L), Mono (13 µg/L); Colorado: Alamosa (36.9 µg/L), Lincoln (23.4 µg/L), Rio Grande (23.8 µg/L); Idaho: Payette (14.4µg/L), Washington (17 µg/L); Illinois: De Witt (17.1 µg/L), Gallatin (22 µg/L); North Dakota: Divide (13.6 µg/L), Lamoure (14.9 µg/L), Ramsey (17.9 µg/L); New Mexico: Bernalillo (14.1 µg/L), Sandoval

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concentrations ≥ 10 $\mu\text{g/L}$ were combined with census data for 1950 to 1999, there were approximately 51.1 million person-years of exposure to drinking water arsenic at levels of ≥ 10 $\mu\text{g/L}$, 8.2 million at levels of ≥ 20 $\mu\text{g/L}$, and 0.9 million at levels of ≥ 50 $\mu\text{g/L}$. More recent information on arsenic detection in drinking water is available from a database of testing results from water utilities maintained by the Environmental Working Group. The most recent report, containing results from 2004 – 2009, found that 1,724 water systems serving more than 11 million people had arsenic test results that exceeded the EPA standard (Environmental Working Group 2009).

As part of the CONTAM Panel evaluation, EFSA issued a call for data on the occurrence of arsenic in food commodities and received more than 100,000 results from 15 countries in Europe. The vast majority of the data, $\sim 98\%$, was reported as total arsenic with the highest levels found in fish and seafood, food products or supplements based on algae (especially hijiki), and cereal and cereal products, with particularly high concentrations in rice grains and rice-based products, and bran and germ.³ The relative proportion of inorganic arsenic in fish and seafood is small and tends to decrease as the total arsenic content increases, and the ratio may vary depending on the seafood type. The major form of organic arsenic found in fish and seafood, arsenobetaine, is generally assumed to be of no toxicological concern (EFSA 2009). Other relevant forms of organic arsenic found in seafood include arsenosugars and arsenolipids, complex compounds that are metabolized in the human body to several metabolites including DMA (Francesconi *et al.* 1997; Francesconi *et al.* 2002; Schmeisser *et al.* 2006; Taleshi *et al.*). Tao and Bolger (1998) assumed that approximately 10% of the total arsenic in seafood is inorganic and 100% is assumed to be in the inorganic form for all other foods, except mushrooms that can contain arsenosugars or arsenobetaine. The ATSDR also described seafood as a main dietary source of arsenic, contributing to an estimated 76 – 96% of total arsenic from the diet (ATSDR 2007). In infants, seafood and rice products are major sources of exposure, contributing 42% and 31% respectively to total dietary intake (ATSDR 2007).

The 2003-2004 NHANES includes measurements of total urinary arsenic and seven arsenic species, including four inorganic-related forms (arsenic acid, arsenous acid, and the methylated metabolites produced in the body, DMA(V) and MA(V), and three organic forms (arsenocholine, trimethylarsine oxide, and arsenobetaine) from 2557 participants aged 6 years and older (Caldwell *et al.* 2009). The major contributors to total arsenic levels were arsenobetaine (main source of arsenic in seafood) and dimethylarsinic acid. Other forms of arsenic were detected less often: arsenic acid, arsenous acid, arsenocholine, and trimethylarsine oxide were detected in only 7.6%, 4.6%, 1.8%, and 0.3% of the population. MA was detected in 35% of participants. The limits of detection for arsenic acid [1.0 $\mu\text{g/L}$], arsenous acid [1.2 $\mu\text{g/L}$] and MA [0.9 $\mu\text{g/L}$], however, were possibly too high for a population exposed to low-moderate levels of inorganic

(17 $\mu\text{g/L}$), Socorro (32.2 $\mu\text{g/L}$); Nevada: Churchill (90 $\mu\text{g/L}$), Esmeralda (25.6 $\mu\text{g/L}$), Lander (17.3 $\mu\text{g/L}$), Lincoln (15.7 $\mu\text{g/L}$), Lyon (21.3 $\mu\text{g/L}$), Nye (13.6 $\mu\text{g/L}$); Oklahoma: Canadian (26.9 $\mu\text{g/L}$), Custer (13 $\mu\text{g/L}$); Texas: Andrews (33.6 $\mu\text{g/L}$), Borden (22 $\mu\text{g/L}$), Gaines (12.1 $\mu\text{g/L}$), Hudspeth (11.6 $\mu\text{g/L}$), Jim Hogg (77.9 $\mu\text{g/L}$), Karnes (15.6 $\mu\text{g/L}$), Yoakum (11.7 $\mu\text{g/L}$); Utah: Summit (12.6 $\mu\text{g/L}$)

³ These findings are consistent with results from U.S. based market basket surveys (Schoof *et al.* 1999)

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arsenic. For comparison, the limits of detections for those species in other epidemiologic studies were ≤ 0.5 $\mu\text{g/L}$ (Chen *et al.* 2002; Lindberg *et al.* 2006; Navas-Acien *et al.* 2009b). The geometric mean levels of total urinary arsenic, arsenobetaine, and DMA were 8.3, 1.55, and 3.71 $\mu\text{g/L}$ (Table 4). DMA, a methylated metabolite of inorganic arsenic, was the major contributor to total arsenic at lower exposure levels (< 20 $\mu\text{g/L}$), with a median contribution of 53.8%. However, at higher levels of total arsenic (≥ 20 $\mu\text{g/L}$), the organic form associated with seafood consumption, arsenobetaine, was the major form, with a median contribution of 43.4% at 20-49 $\mu\text{g/L}$ total arsenic and 62.7% at ≥ 50 $\mu\text{g/L}$ total arsenic (Caldwell *et al.* 2009). Based on standard reverse dosimetry assumptions that all ingested arsenic is excreted and that an average of 1 liter of urine is excreted a day (Kile and Christiani 2008), a urinary arsenic concentration of 8.3 $\mu\text{g/L}$ in a 70-kg individual would relate to an ingested dose of ~ 0.12 $\mu\text{g/kg-d}^4$. Urinary arsenic is considered a biomarker for short-term exposure because the half-life of arsenic is ~ 3 -days (Kile and Christiani 2008).

⁴ This estimate does not take into account fecal excretion nor differences in methylation or metabolic capacity based on sex or age.

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Table 4. Urinary arsenic geometric mean and selected percentiles of urinary concentrations (in µg/L) for the US population, NHANES 2003-2004 (Caldwell et al., 2009)

	Geometric mean and selected percentiles (95% confidence interval)														
	All (n = 2557 – 2568)					6-11 years (n = 290 – 292)					≥ 20 years (n = 1542 – 1548)				
	geometric mean	25 th	50 th	75 th	95th	geometric mean	25 th	50 th	75 th	95th	geometric mean	25 th	50 th	75 th	95th
Total arsenic	8.30	4.1	7.7	16	65.4	7.08	4.40	6.7	10.7	46.9	8.41	3.9	7.9	17	66.1
Sum of inorganic-related species*	–	–	6.0	–	18.9	–	–	6.0	–	14.7	–	–	5.9	–	19.4
Arsenic acid (inorganic)	–	–	–	–	1.10	–	–	–	–	–	–	–	–	–	–
Monomethylarsonic acid (inorganic)	–	–	–	–	2.40	–	–	–	–	–	–	–	–	–	–
Dimethylarsinic acid (inorganic)	3.71	2.0	3.9	6.0	16.0	3.73	1.90	3.9	5.9	12.0	3.69	2.0	3.7	6.0	15.8
Arsenobetaine (organic)	1.55	<LOD	1.0	5.1	35.0	–	<LOD	<LOD	1.7	29.7	1.74	<LOD	1.3	6.1	35.2

– , not calculated because the proportion of results below the limit of detection (LOD) was too high to provide a valid result

*Sum of arsenic acid, arsenous acid, dimethylarsinic acid, and monomethylarsonic acid

The LOD for urinary arsenic was 0.74µg/L

From Caldwell et al. (2009)

1.3 Epidemiological Studies on Diabetes

The first epidemiological studies reporting associations between arsenic and diabetes were published in mid-1990s. These early studies were conducted in populations exposed to high levels of arsenic in drinking water in Taiwan and Bangladesh or in copper smelter and glass workers in the US and Europe exposed to arsenic by inhalation or other pathways. Literature reviews of these studies have concluded that arsenic exposure was most consistently associated with diabetes in the “high” arsenic areas like Taiwan and Bangladesh where water concentrations $>150 \mu\text{g/L}$ have been reported; results from worker studies were more inconsistent (Chen *et al.* 2007; Longnecker and Daniels 2001; Navas-Acien *et al.* 2006; Tseng *et al.* 2002). Over the last decade additional epidemiologic studies have tended to focus on the association between “low-to-moderate” arsenic exposure and diabetes in the general population (e.g., NHANES data in the US) or regions with relatively elevated levels of arsenic that are lower than levels reported in the HAA studies in Taiwan or Bangladesh. At the time of the last comprehensive review by Navas-Acien *et al.* (Navas-Acien *et al.* 2006), the “low-to-moderate” arsenic studies (typical drinking water levels of $\sim 10\text{--}149 \mu\text{g/L}$) did not show an overall increased risk of diabetes with higher arsenic exposures (relative risks ranged from 0.65 to 1.09; median of 0.95). However, since 2007, twelve epidemiological studies in populations with “low-to-moderate” exposure have been published (Afridi *et al.* 2008; Chen *et al.* 2010; Coronado-Gonzalez *et al.* 2007; Del Razo *et al.* submitted; Ettinger *et al.* 2009; Meliker *et al.* 2007; Navas-Acien *et al.* 2008, 2009a; Steinmaus *et al.* 2009a; Steinmaus *et al.* 2009b; Wang *et al.* 2009; Wang *et al.* 2007) that were not considered in the review by Navas-Acien *et al.* (2006) or other reviews.

Arsenic was once used as an anti-diabetic drug with 9 reports of its use for this purpose prior to the discovery of insulin, including a publication from 1892 (Helmstadter 2007). However, it did not become an established treatment for diabetes as it did for some tropical diseases, skin lesions, or leukemias. Hyperglycemia is considered one of the more common side effects in patients treated with arsenic trioxide for acute promyelocytic leukemia (APL) (Rust and Soignet 2001). One case of grade 4 pancreatitis and several cases of hyperglycemia were observed in children and adolescents participating in a phase 1 trial and pharmacokinetic study of arsenic trioxide as a therapeutic agent for refractory or relapsed acute leukemia (Fox *et al.* 2008).

Recently, an excess of pancreatic cancer was reported in adults who were exposed to high levels of inorganic arsenic as neonates from ingesting contaminated milk powder in Japan during the summer of 1955 (Yorifuji *et al.* 2010). Using vital statistics data for 1970—2006, Yorifuji *et al.* (2010) compared cancer mortality in the 5-year birth cohort that included subjects born before the milk poisoning (exposed group) to a similar cohort born after the incident (control group). Such an analysis led to strong dilution as the number of clinically poisoned subjects in the 5-year “exposed” birth cohort may represent only 4% of the cohort. Despite this dilution, the mortality ratio for pancreatic cancer during 1970—2006 compared with the next younger 5-year birth cohort born within the same 5 year calendar period was elevated (MR – 1.52; 95% CI: 1.23—1.87) as was the mortality ratio when the birth cohort representing the

exposed group was compared with the same 5-year age group born during the subsequent 5-year calendar period (MR = 1.41; 95% CI: 1.08–1.83).

1.3.1 Occupational Exposure

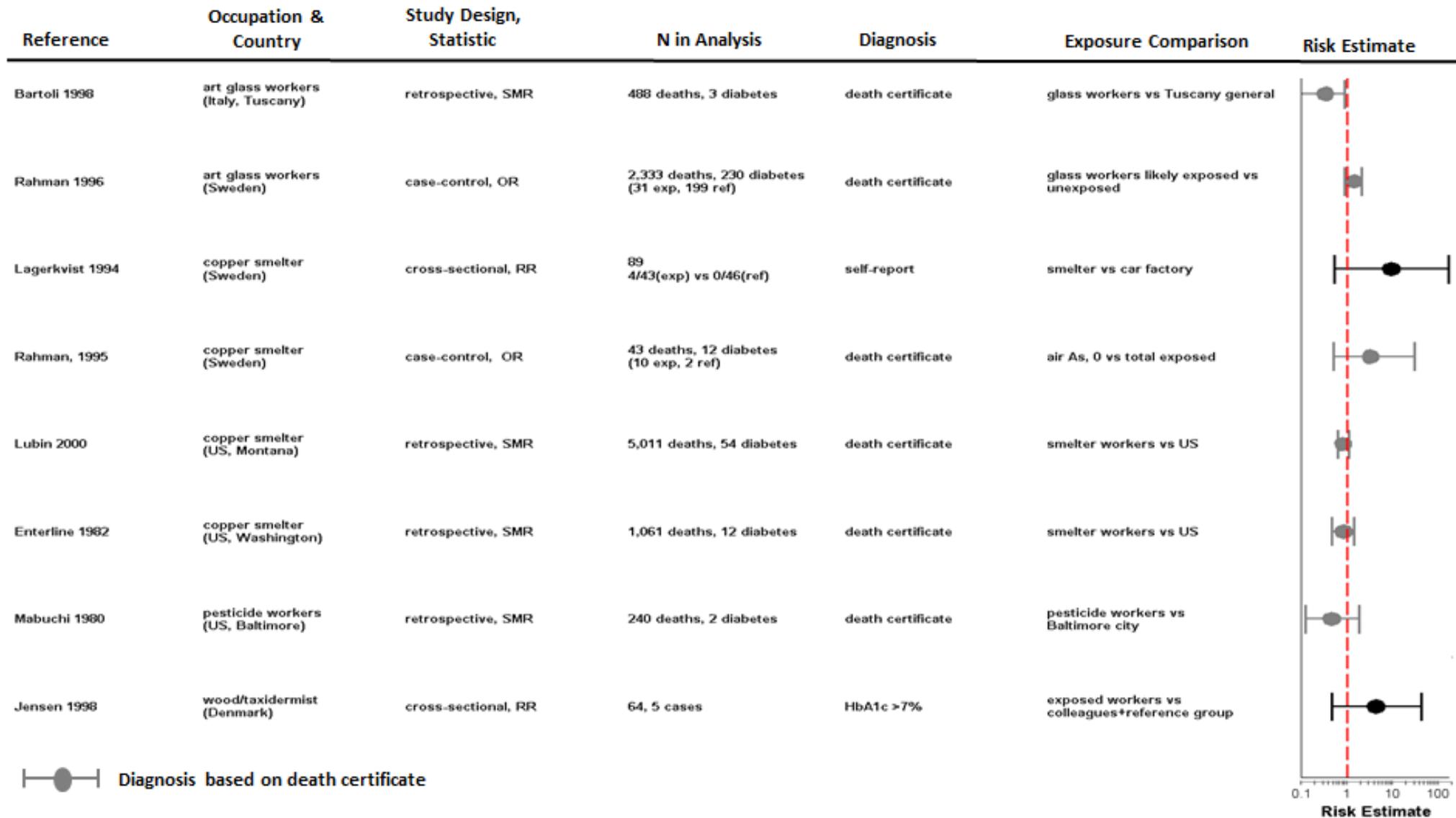
No consistent association was observed between arsenic exposure and type 2 diabetes in the 8 occupational studies identified for this evaluation, published between 1980 and 2003 (Figure 1 and Appendix Table A). This is the same conclusion reached in previous reviews of the worker studies (Chen *et al.* 2007; Longnecker and Daniels 2001; Navas-Acien *et al.* 2006). These studies were mostly based on workers exposed via inhalation in the copper smelter and glass industries or people living near these types of facilities and were likely exposed to other organic and inorganic pollutants. In general these studies were not designed to look at diabetes and it was one of many causes of death examined. Diabetes was defined based on death certificates in 7 studies, self-reported diabetes in one study (Lagerkvist and Zetterlund 1994) and glycosylated hemoglobin in another study (Jensen and Hansen 1998). The range of association values was an SMR of 0.34 (95% CI 0.09-0.88) in Bartoliet *al.* (1998) to an OR of 9.61 (95% CI: 0.53-173) in Lagerkvist and Zetterlund (1994) with a median value of 1.13 for the studies in workers. A similar pattern was observed when only the studies of copper smelter workers (the most commonly evaluated occupation, represented in 4 studies) were considered with a range of 0.83 (95% CI 0.63-1.08) to 9.61 (95% CI: 0.53-173)(Enterline and Marsh 1982; Lagerkvist and Zetterlund 1994; Lubin *et al.* 2000; Mabuchi *et al.* 1980).

There are a number of limitations to the occupation literature, including the bases of exposure and disease categorization, especially with respect to use of death certificates to assess diabetes, as well as small sample sizes. Seven of the nine studies relied on death certificates as the basis of diagnosing diabetes which is likely to severely underestimate the prevalence/incidence of people with the disease (Tseng 2006). In a US-based study designed to characterize the sensitivity and specificity of death certificates for diabetes, Cheng *et al.*, (2008) found that diabetes was listed as a direct or contributing cause of death on only 6.2% death certificates for adults who were known to have diabetes. With the exception of a study by Rahman *et al.* (1996) which reported 230 diabetes deaths in ~2330 workers, the studies were generally of small sample size or relatively few cases of diabetes or deaths attributed to diabetes, i.e., seven of the nine studies had fewer than 17 cases. The degree of arsenic exposure was typically based on occupation, although the study by Rahman *et al.* (1995) calculated ORs based on arsenic levels in air. Reference groups were workers in occupations with less arsenic exposure or the general population living in the same or similar geographic region. Reliance on occupation as a measure of arsenic exposure may be problematic. For example, in the study by Jensen and Hansen (1998) urinary arsenic levels in occupations considered exposed (e.g., taxidermists, wood workers, etc.) overlapped with levels measured in the reference group (11.5–294.5 versus 6.0–44.0 nmol As/mmol creatinine).

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Figure 1. Occupational studies of arsenic and diabetes



Based on studies described in [Appendix Table A](#). SMR = standardized mortality ratio, RR = relative risk, OR = odds ratio, PR = prevalence ratio

1.3.2 Environmental Exposure, High Arsenic Areas

Higher concentrations of arsenic in groundwater or drinking water are reported in Bangladesh, Taiwan (China), West Bengal (India), Peoples Republic of China (Xinjiang and Inner Mongolia), regions of Chile, North Mexico, Argentina, southwestern Finland, Vietnam, and parts of the US (California, Utah, Nevada, Washington, and Alaska) (IPCS 2001). The definition of what constitutes a “higher” concentration of arsenic is relative and some regions can be considered very high, i.e., >300 µg/L drinking water was common in areas where the carcinogenic effects of arsenic were established for humans (Meliker *et al.* 2007). Residents living in high arsenic areas (HAAs), also referred to as arsenic endemic areas, may have arsenic-related health conditions such as blackfoot disease (gangrene of the extremities) or keratosis. Well water concentrations of arsenic associated with blackfoot disease range from 170-800 ppb (ATSDR 2007). Other regions are considered to have elevated arsenic levels by virtue of being higher than a regulatory level, currently set at 10µg/L by the US EPA. For the purposes of this evaluation, studies are categorized as “high exposure” if they are conducted in regions with water arsenic concentrations above ~150 µg/L and “low-to-moderate” if typical drinking water levels are ~10–149 µg/L.

Not every study of arsenic in Taiwan or Bangladesh should be considered an HAA study. The hair arsenic levels reported by Wang *et al.* (2007) from residents of central Taiwan are lower compared to hair measurements from people with blackfoot disease living in other parts of Taiwan or compared to people in Mexico living in arsenic endemic areas with skin effects associated with arsenic (i.e., hyper- or hypopigmentation, hyperkeratosis). One Bangladesh report from the Health Effects of As Longitudinal Study (HEALS) by Chen *et al.* (2010) included exposure categories that could be considered “low to moderate” (8.1 to 91.7 µg/L water) and “high” (176.2 to 864 µg/L water) and is therefore discussed in both sections.

With the exception of a 2010 study by Chen *et al.* (2010), seven of eight HAA studies conducted in Taiwan or Bangladesh reported positive associations between arsenic and diabetes⁵ (Figure 2 and Appendix Table A) (Lai *et al.* 1994; Nabi *et al.* 2005; Rahman *et al.* 1998; Rahman *et al.* 1999; Tsai *et al.* 1999; Tseng *et al.* 2000b; Wang *et al.* 2003). In Taiwan the clinical features of diabetes mellitus have been described as more consistent with type 2 diabetes than type 1 because cases did not develop diabetic ketoacidosis or require insulin treatment during initial follow-up (Tseng 2004). Two of the reports from Taiwan, carried out in blackfoot disease endemic villages, used different approaches to examine the same population. The study by Lai *et al.* (1994) was cross-sectional and the study by Tseng *et al.* (2000) was a prospective follow-up of the same cohort. Lai *et al.* (1994) had three exposure categories (unexposed reference group, < 15 ppm-yrs, and ≥ 15 ppm-yrs) and found a dose-response relation between cumulative arsenic exposure (CAE) and the prevalence of diabetes mellitus after adjustment for multiple risk factors, including age and BMI. The multivariate-adjusted odds ratios were 6.61 (95% CI 0.86, 51.0) and 10.05 (95% CI 1.30, 77.9). Tseng *et al.* (2000b) followed the same cohort of individuals without diabetes-mellitus and compared the incidence of diabetes mellitus in

⁵ The Nabi *et al.* (2005) study reported an increase in the prevalence of diabetes in patients with arsenicosis (10%) compared to no arsenicosis (3.5%), but did not report any statistical analysis of this ratio.

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people with high arsenic exposure (CAE \geq 17.0 mg/L-yrs) to those with low arsenic exposure (CAE <17.0mg/L-yrs). The cohort was followed biannually for two years. After adjusting for age, sex and BMI, those with CAE \geq 17.0mg/L-yrs had a two times higher risk of developing diabetes mellitus: 2.10 (95% CI 1.1, 4.2).

A study not presented in [Figure 2](#) by Chiu et al. (2006) looked at the pattern of SMRs for diabetes in Taiwan during the period of 1971-2000, after the replacement of artesian well water with municipal water for drinking and cooking that occurred between 1966 and 1975. Mortality due to diabetes seemed to decrease in women but not men after improvement in the drinking water supply system, leading the authors to suggest that the association between arsenic and diabetes might be causal for women but not men. This assessment was criticized by Tseng et al. (2006) for a number of reasons, including the use of death certificates to ascertain diabetes, failing to account for the migration out of arsenic-endemic regions that occurred in Taiwan during the period covered in the SMR analysis, and not including SMR data for the period prior to improvement of drinking water supply.

The association between arsenic and diabetes is not as consistent in studies conducted in Bangladesh. Two studies by Rahman et al. (1998; 1999) reported statistically significant positive associations. Another study by Nabi et al. (2005) reported an increase in the prevalence of diabetes in patients with arsenicosis (10%) compared to individuals without arsenicosis (3.5%), but did not report any statistical analysis of this ratio. However, the most recent and largest study by Chen et al. (2010) did not find any significant associations between arsenic and self-reported diabetes, glucosuria⁶ or HbA1c levels in a population-based cross sectional study of 11,319 Bangladeshi men and women participating in the Health Effects of As Longitudinal Study (HEALS). In the highest exposure categories the adjusted ORs for diabetes prevalence based on self-report of physician diagnosis was 1.11 (95% CI 0.73-1.69) in relation to time weight water arsenic (TWA) of 176.2–864 μ g/L and 0.93 (95% CI 0.59-1.45) for urinary arsenic concentrations >205 μ g/L (findings at lower arsenic levels are described below in the “low-to-moderate” exposure section). Findings from this study, other epidemiological studies, and studies in laboratory animals and *in vitro* model systems led Chen et al.(2010) to conclude that arsenic may only be associated with diabetes in populations with prolonged exposure to very high levels of water arsenic (>500 μ g/L).

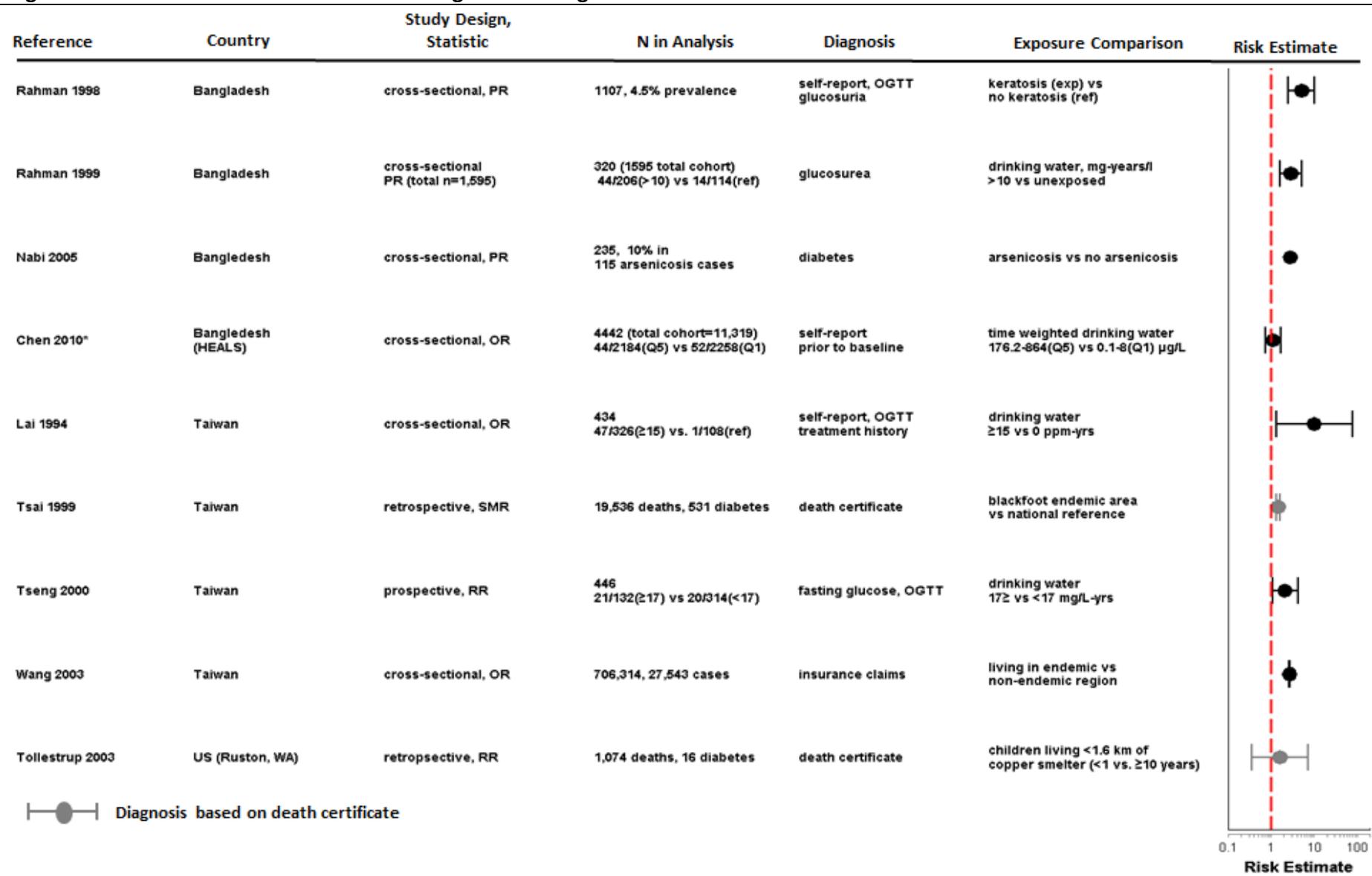
In addition to the eight studies in workers described above, one copper smelter exposure study included an assessment of children. Tollestrup et al. (2003) looked at the relationship between arsenic exposure and diabetes as a cause of death in people who lived within 1.6 km (1 mile) of a copper smelter for at least 2 years prior to the age of 14 and did not detect a significant association [OR of 1.6 (95% CI: 0.36-7.16) between children living there for \geq 10 years versus <1 year of residency.

⁶ Glucosuria is not considered a reliable estimate of blood glucose (Goldstein *et al.* 2004)

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Figure 2. Studies of arsenic and diabetes in regions with high arsenic areas



Based on studies described in [Appendix Table A](#)

*Similar ORs were reported by Chen et al (2010) based on urine As levels, both creatinine adjusted and unadjusted. The OR included in Figure 2 are from the highest exposure category (176.2–864 µg/L water). Findings from this study for exposure categories considered “low to moderate” are presented in [Figure 4](#).

1.3.3 Environmental Exposure, Low-to-Moderate Arsenic Areas

Over 15 epidemiology studies were identified that looked at the issue of diabetes, glycemic control, or metabolic syndrome in people living in regions considered to be low to moderate arsenic exposure (10 to ~150 µg/L iAs in drinking water) (Figure 4, Figure 5, and Appendix Table A). Six studies reported statistically significant associations between inorganic arsenic exposure and diabetes or glucose tolerance, two in Mexico (Coronado-Gonzalez *et al.* 2007; Del Razo *et al.* submitted) and four in the United States (Ettinger *et al.* 2009; Meliker *et al.* 2007; Navas-Acien *et al.* 2008, 2009a).

A consistent association was found in three studies of populations with higher BMIs who also live in regions with “moderate” iAs (Cobo and Castineira 1997; Del Razo *et al.* submitted; Ettinger *et al.* 2009). In contrast, a large cross-sectional study conducted in Bangladesh, where people are much leaner, did not observe any association between diabetes and arsenic. A greater degree of inconsistency is found in studies conducted in the US of exposures characterized as general population levels or at the low end of the “moderate” category with reports of positive associations (Ettinger *et al.* 2009; Meliker *et al.* 2007; Navas-Acien *et al.* 2008, 2009a) and no association (Lewis *et al.* 1999; Steinmaus *et al.* 2009a; Steinmaus *et al.* 2009b; Zierold *et al.* 2004). Some of the differences in findings across the studies could be due to variation in sample size and methods for diagnosing diabetes (e.g., death certificates versus self-report or blood glucose) or assessing arsenic exposure (e.g., urine levels versus drinking water surveys).

Four publications addressed general population exposure levels by using NHANES data to evaluate cross-sectional relationships between urinary arsenic and diabetes⁷ (Navas-Acien *et al.* 2008, 2009a; Steinmaus *et al.* 2009a; Steinmaus *et al.* 2009b). Differences in the approaches used by these two research groups led to findings of either significant associations (Navas-Acien *et al.* 2008, 2009a) or no association (Steinmaus *et al.* 2009a; Steinmaus *et al.* 2009b) (Figure 5). The initial report by Navas-Acien *et al.* (2008) analyzed data from the 2003-2004 NHANES and reported a 3.58-fold increase (95% CI, 1.18-10.83) in prevalent diabetes at the 80th (16.5 µg/L) versus the 20th (3.0 µg/L) percentiles of total urinary arsenic level after adjusting for diabetes risk factors and markers of seafood intake to remove the contribution of organic arsenicals to total urine arsenic. Additional adjustment for family history of diabetes, use of hormone therapy, and use of dietary supplements did not appreciably change the results [author’s reply to Tseng (2008)]. This report attracted considerable scientific (Kile and Christiani 2008; Tseng 2008) and public attention (Brophy 2008; CNN 2008; Voiland 2008) and initiated a series of discussions on the most appropriate statistical strategies to analyze NHANES data for associations between arsenic and diabetes (Longnecker 2009; Navas-Acien *et al.* 2009a; Steinmaus *et al.* 2009a; Steinmaus *et al.* 2009b). In brief, the discussions focused on (1) the most appropriate strategy to adjust total urine arsenic for contributions due to the less toxic organic forms of arsenic associated with seafood consumption, and (2) whether or not urine creatinine should be included as a variable in the multiple regression analysis given that

⁷Diagnosed based on fasting serum glucose level ≥126 mg/dL, or self-reported physician diagnosis.

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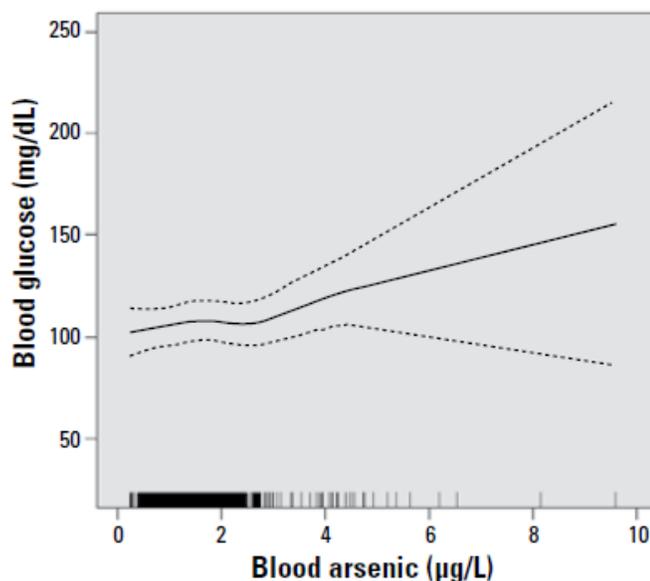
(version updated January 3, 2011)

changes in creatinine are associated with both diabetes and arsenic exposure. These issues are discussed in more detail below.

One study focused on risk of impaired glucose tolerance during pregnancy. Ettinger et al. (2009) looked at whether arsenic may contribute to the development of diabetes during pregnancy in 532 women living near the Tar Creek Superfund site in Ottawa, Oklahoma, an area where 25% of the drinking water samples contain more than 10 $\mu\text{g/L}$ arsenic (based on data from USGS 2000). Blood arsenic levels were

associated with an increased risk of impaired glucose tolerance test at 24-28 weeks gestation comparing the highest to the lowest quartile of exposure (OR 2.8, 95% CI 1.1-6.9). There was a statistically significant trend in risk of impaired OGTT by increasing quartile of exposure (p -trend = 0.008). Figure 3 shows the dose-response relationship between arsenic and blood glucose from a 1-hour OGTT presented on a continuous scale after adjustment for variables such as pre-pregnancy BMI, etc. Hair arsenic levels were available in a smaller subset of women, about 30% of the total sample. There was no association with blood glucose when the analysis was based on hair arsenic levels, which did not correlate well with blood arsenic measurements (Spearman's $\rho = -0.13$, $p=0.08$). This may also suggest that the association is stronger when based on a current measure of exposure (urine rather than hair).

Figure 3. Dose-response relationship between blood As and blood glucose in a 1-hour OGTT in 532 pregnant women (Ettinger et al., 2009)



Adjusted dose-response relationship for the effect of blood arsenic ($\mu\text{g/L}$) on blood glucose (mg/dL) from a generalized additive model with penalized splines ($df = 3$, $p = 0.01$) adjusted for age (centered), centered age-squared, pre-pregnancy BMI, Native-American race/ethnicity, use of Medicaid insurance, and married or living with partner; weighted by the inverse of the variance of exposure variable ($n = 455$). Dashed lines represent 95% CIs.

From Figure 1 in Ettinger et al. (2009)

Two studies in the US used death certificates to look at diabetes as a cause of death in relation to levels of arsenic in drinking water. The largest study, based on almost 80,000 death certificates, found elevated SMRs for residents of six counties in southeastern Michigan with higher levels of well water arsenic compared to other counties in the state (Meliker *et al.* 2007). The population-weighted mean arsenic in the water samples collected from the 6 counties was 11 $\mu\text{g/L}$ compared to 2.98 $\mu\text{g/L}$ in the rest of the state. The SMRs for men and women were 1.28 (99% CI 1.18-1.37) and 1.27 (99% CI 1.19-1.35), respectively (Figure 4). No increase in SMRs for men or women were reported in a smaller study by Lewis et al. (1999) based on

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analysis of 2,203 death records from a cohort of residents of Milliard County, UT. In this study, the median drinking water levels of arsenic found in public and private samples ranged from 14 to 166 µg/L.

Two studies have looked at the association between arsenic and diabetes in arsenic endemic regions of Mexico, the Coahuila region in Coronado-Gonzalez et al. (2007) and the Zimapan and Lagunera regions in Del Razo et al. (submitted). The levels of water arsenic in these regions are generally lower⁸ than those reported in the past for Bangladesh or Taiwan (>300 µg/L), but higher than regions in the US considered “moderate” exposure based on having drinking water levels above the EPA standard. Coronado-Gonzalez et al. (2007) conducted a case-control study with 200 diabetics and 200 non-diabetics and found a two-fold higher risk of diabetes [adjusted OR of 2.16, 95% CI 1.23–3.79] in subjects with urine levels of total arsenic of 63.5–104 µg/g creatinine compared to subjects with levels <63 µg/g creatinine. The risk was higher in subjects in the highest exposure category, 104 µg/g creatinine, with an adjusted OR of 2.84, 95% CI 1.64–4.92).

In a small study involving 257 subjects, Del Razo et al. found that drinking water arsenic levels of ≥125 µg/L were significantly associated with diabetes OR = 5.01 (95% CI 1.02–24.17), based on subjects having fasting blood glucose ≥126 mg/dL (personal communication on unpublished data by Miroslav Styblo et al., August 6, 2010). The risk of diabetes measured by fasting blood glucose and 2-hour oral glucose tolerance was positively associated with iAs in drinking water (OR 1.14–1.15 per 10 ppb, $p \leq 0.002$) and with the concentration of DMAIII in urine (OR 1.04–1.05 per ng As/mL, $p \leq 0.05$). Higher urine levels of total arsenic or MAIII were not significantly associated with diabetes in this study. The authors also found negative associations between fasting plasma insulin and HOMA-IR (homeostasis model assessment of insulin resistance), and water arsenic (β -0.21 and -0.16, respectively, $p < 0.001$). The overall finding of elevated risk of diabetes without indications of insulin resistance led the authors to suggest that the type of diabetes related by arsenic exposure may differ from type 2 diabetes, which is characterized by insulinemia and insulin resistance. The pattern observed with inorganic As exposure of higher fasting blood glucose with lower fasting plasma insulin suggests an impairment of β -cell function. This research group also examined the role of genetic polymorphism for arsenic (+3 oxidation state) methyltransferase (AS3MT) in modulation of the metabolism and health effects of inorganic arsenic. They found that carriers of the AS3MT/287Thr variant generally had higher fasting blood glucose, 2-hour fasting blood glucose, and HbA1c levels in blood. Importantly, this genotype was also associated with higher concentrations of DMAIII in urine. This suggests that the AS3MT/287Thr variant is associated with an increased risk of developing diabetes, possibly due to a greater capacity to convert iAs to DMAIII (personal communication on unpublished data by Miroslav Styblo et al., August 6, 2010).

⁸ Sites in Coahuila, Mexico were selected based on prior findings of 20–400 µg/L in sources of water (Coronado-Gonzalez et al. 2007). The average level of arsenic in water in the Del Razo et al. (submitted) was 42.9 µg/L and ranged from 3.1 to 215.2 µg/L.

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A recent large study in Bangladesh did not observe any significant associations between arsenic levels in urine or time-weighted water arsenic (TWA) and increased prevalence of diabetes mellitus in a population-based cross sectional study of 11,319 Bangladeshi men and women participating in the Health Effects of As Longitudinal Study (HEALS). TWA was categorized into quintiles and the range of arsenic exposure categories compared to the reference group included both “low to moderate” (8.1-91.7 µg/L TWA) and “high” (176.2-864 µg/L TWA). Results for the “high” exposure category are discussed above. More than 90% of cohort members were exposed to drinking water containing less than 300 µg/L. The average duration of well use for wells with a known arsenic concentration accounted for 25 percent of lifetime for both genders (10.0 years for males and 8.3 years for females). No association was detected whether arsenic exposure was based on TWA arsenic or urine arsenic. The lack of association based on urine arsenic was apparent in multiple regression models that included or excluded urine creatinine as an adjustment. Similarly, no associations were observed between TWA or urinary arsenic and prevalence of urine glucosuria (based on 231 diabetes cases and 10,497 non-cases) or HbA1c levels (based on 45 diabetes cases and 1999 non-cases). Findings from this study findings, other epidemiological studies, and studies in laboratory animals and *in vitro* model systems (discussed below) led Chen et al. (2010) to conclude that arsenic may only be associated with diabetes in populations with prolonged exposure to very high levels of water arsenic (> 500 µg/L).

Although this study was quite large, the number of participants reporting a diagnosis of diabetes prior to baseline was 241 or ~2% of the total cohort. Diabetes diagnosis was based retrospectively on self-report during first two-year follow-up because baseline interview did not include questions on diabetes status. In addition, glucosuria is not an accepted diagnostic tool for diabetes (Goldstein *et al.* 2004)⁹. This temporal mismatch in the assessment of exposure and disease could result in misclassification of exposure. It is also unclear whether HbA1c has the same interpretive value in Bengali compared to other populations. The median HbA1c level in diabetes in the Chen et al study was 6.8%.

With respect to body weight, the BMI levels in this population are lower than in the US (BMI of <22 in ~80% of study participants) and there was no indication that arsenic exposure was positively associated with having a higher BMI, defined as ≥ 22.1 .¹⁰ The BMI of participants in the Bangladeshi study by Chen et al. (2010) was quite low, median of 22.5 and 19.2 in diabetics and non-diabetics, respectively. By way of comparison, 68% of study participants included in the analysis of NHANES 2008 had a BMI of ≥ 25 (Navas-Acien *et al.* 2008). In the Mexico studies 34% of participants in Del Razo et al. (Del Razo *et al.* submitted) and ~42–50% of subjects in

⁹ Findings from De Del Razo et al. (submitted) suggest that HbA1C may be a less sensitive indicator of diabetes compared to other measures such as fasting blood glucose or OGGT. Glucosuria is not considered a very useful indicator of blood glucose (Goldstein *et al.* 2004).

¹⁰ Measures of arsenic exposure were lower in higher BMI categories ($p < 0.01$ from linear regression), e.g., median total urine arsenic of 82 versus 91 µg/L in participants with a BMI of ≥ 22.1 compared to those with a BMI of <18. This finding is consistent with a report that arsenic levels do not seem to be associated with amount of fat tissue (Ronco *et al.* 2010)

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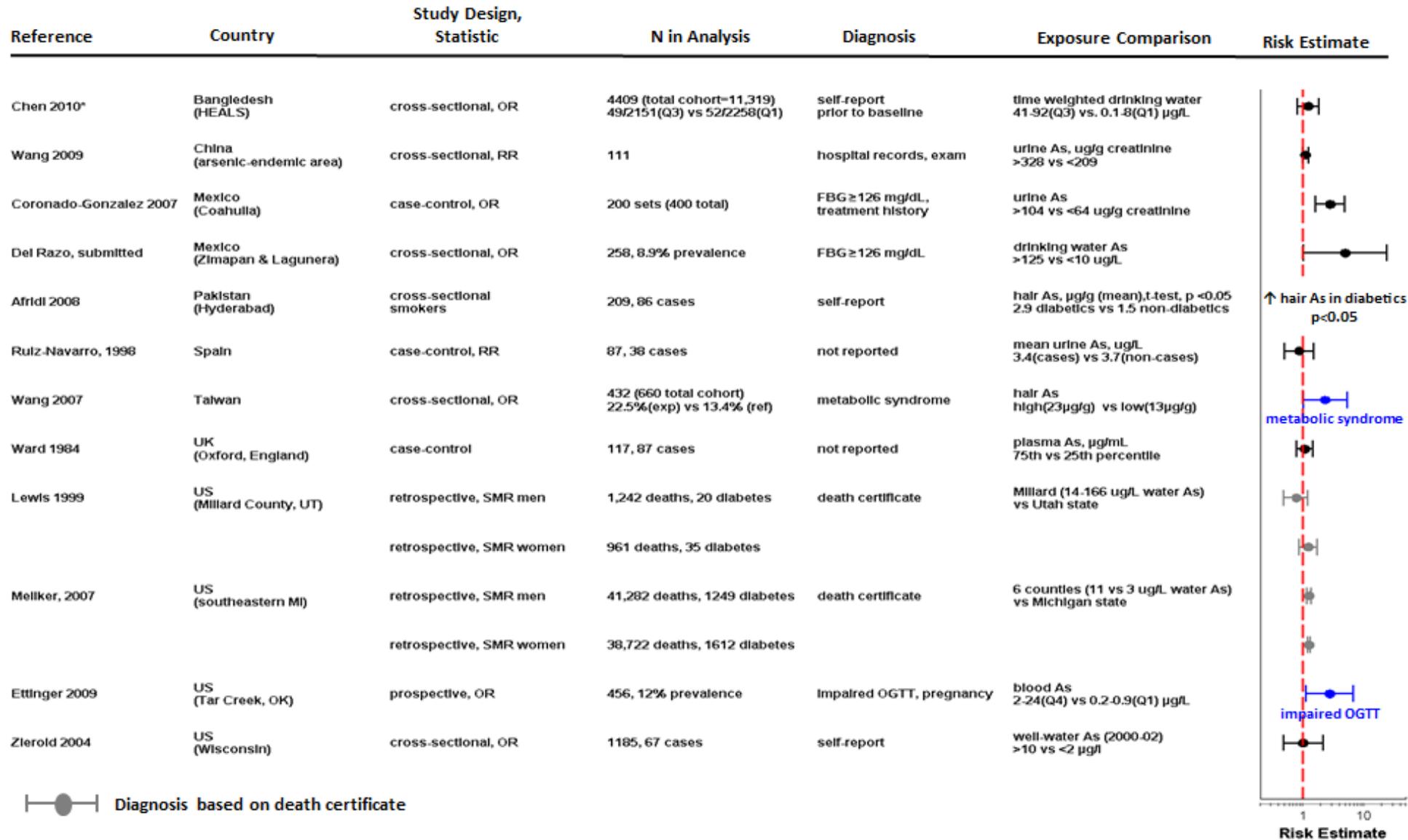
Coronado-Gonzalez (2007) had a BMI >30. Information on BMI was not presented in most of the studies conducted in Taiwan except for Tseng et al. (2000b), average BMI of ~24. As a population the Taiwanese have a higher BMI than Bengali. The prevalence of overweight in Taiwan, defined as a BMI ≥ 24 , has been reported as 30.5% in men and 21.3% in women (Huang 2008). Although the regression models include BMI as a variable, it is unclear whether this is sufficient to account for the biological differences that might exist between very lean people, like residents of Bangladesh, and other populations that with higher BMIs.

One report from Taiwan examined metabolic syndrome as an outcome measure because it is considered a strong predictor for the development of diabetes (Wang *et al.* 2007). Metabolic syndrome was defined as the presence of three or more risk factors: elevated levels of blood pressure, plasma glucose, triglycerides, BMI, and reduced high-density lipoprotein. The authors recruited 660 subjects from a random population of residents in central Taiwan during 2002-2003 and measured their hair arsenic concentrations. When compared to the lowest exposure level group as the reference group, the prevalence of metabolic syndrome increased significantly at the median or 2nd tertile exposure level (OR 2.54, 95% CI 1.20-5.39) and the high exposure (3rd tertile) level (OR 2.35, 95% CI 1.02-5.43), after adjusting for age, gender, occupation and lifestyle.

Two additional case-control studies using subjects from the United Kingdom (Ward and Pim 1984) or Spain (Ruiz-Navarro *et al.* 1998) were also identified in the literature review. Neither of these studies reported any significant association between arsenic and diabetes but the overall utility of the studies is quite limited. First, the studies are likely underpowered statistically to detect an association for what were presumably general population levels of exposure. The total number of subjects evaluated was 117 (87 cases of diabetes) in Ward et al. (1984) and 87 (38 cases of diabetes) in Ruiz-Navarro et al. (1998). In addition, important methodological details are not described in the studies, such as the diagnostic approach used to identify subjects as being diabetic.

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Figure 4. Studies of arsenic and diabetes in regions with low to moderate arsenic areas



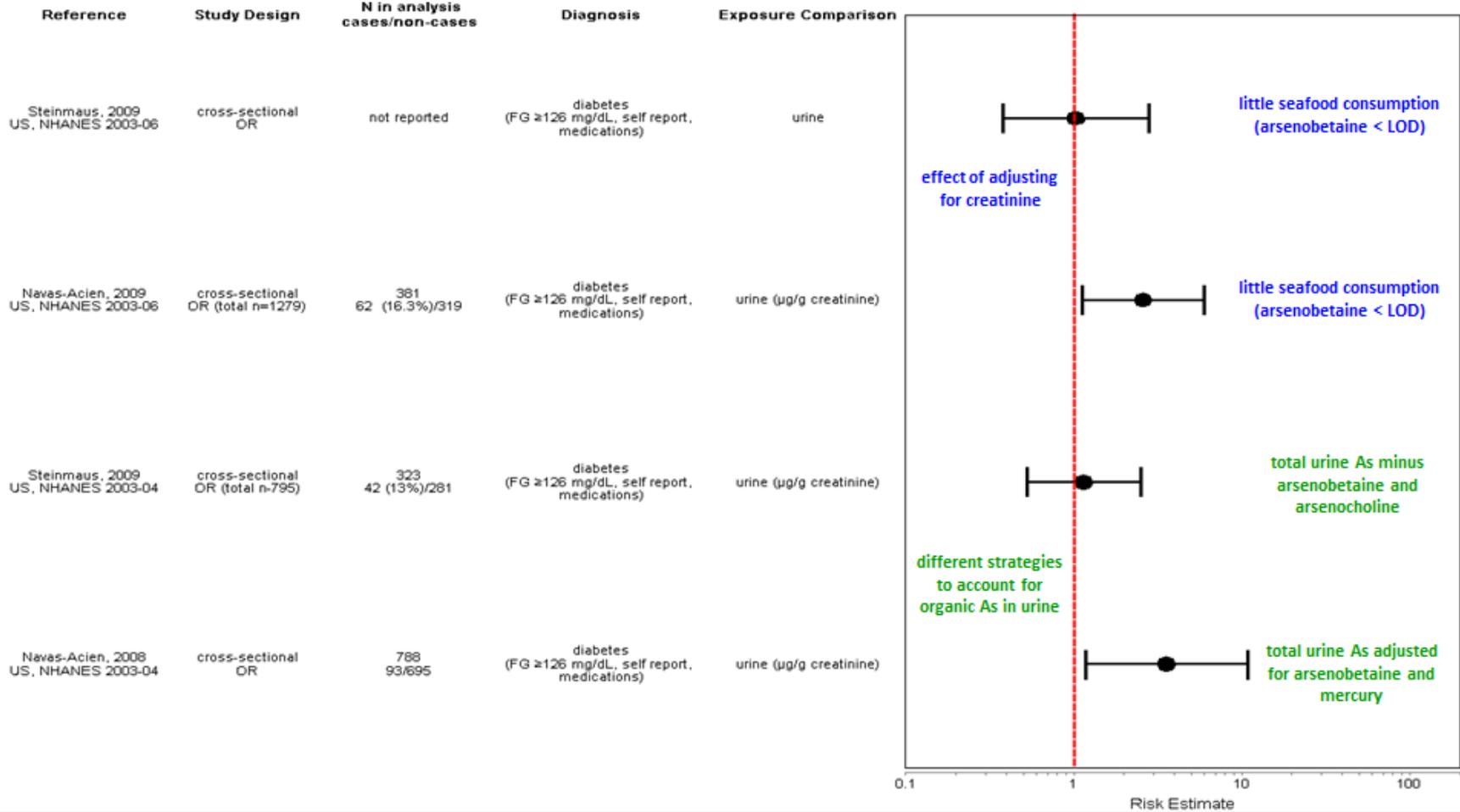
Based on studies described in [Appendix Table A](#)

Similar ORs were reported by Chen et al (2010) based on urine As levels, both creatinine adjusted and unadjusted. The OR included in Figure 3 are from the highest exposure category that falls in the “low to moderate” range (Q3: 41.2–91.7µg/L water). Findings from this study for exposure categories considered “high” are presented in [Figure 2](#)

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Figure 5. Association between arsenic exposure and diabetes in NHANES



1.3.4 Areas of Complexity in the Epidemiology Studies

Accounting for Organic Arsenic

Most human biomonitoring studies report levels of total arsenic which includes inorganic (i.e., arsenite, arsenate) and organic arsenic compounds (mainly arsenobetaine, arsenosugars, and arsenolipids) and their metabolites (Table 4 and Figure 6). It is important to discern how much of the total arsenic measurement is due to intake of inorganic arsenic because organic arsenicals, mostly found in seafood, are generally considered to be of little toxicological significance. In many cases only total urinary levels of arsenic are reported in human biomonitoring studies and it can be challenging to reach conclusions on associations between inorganic arsenic and diabetes or other health measures. This is less of a challenge when study participants are exposed to higher levels of arsenic from drinking water, occupation, or proximity to an industrial or mining site with arsenic contamination. In these cases, urinary arsenic is generally assumed to be mostly from exposure to inorganic arsenic and other exposure to pollutants is likely. However, in studies of the general population like NHANES it is more difficult to identify the portion of urinary arsenic that can be attributed to intake of organic arsenic, mostly due to seafood consumption (Longnecker 2009; Navas-Acien *et al.* 2009a; Steinmaus *et al.* 2009a).

NHANES includes measurement of total arsenic and seven arsenic species four inorganic-related forms (arsenite, arsenate, the methylated metabolites produced in the body dimethylarsinic acid (DMA), and monomethylarsonic acid (MA) and three organic forms (arsenobetaine, arsenocholine, and trimethylarsine oxide). The measurement of total arsenic is a separate chemical analysis and reflects other arsenicals in addition to the 7 species measured, i.e., total arsenic is not the sum of the 7 specific species. With respect to the species measured in NHANES it is important to take into account some issues. First, the species more readily reflecting inorganic arsenic exposure (arsenite, arsenate, and MA) are undetectable in the majority of the general population (Table 4) and cannot be used in epidemiologic studies. Second, although DMA is the major metabolite of inorganic arsenic is also a metabolite of arsenosugars and arsenolipids and therefore reflects both exposures to inorganic and organic forms of arsenic (Figure 6). Third, arsenocholine and trimethylarsine oxide are not the major forms of arsenic found in seafood and are not considered to be significant sources of exposure to organic arsenic. This is supported by the finding that these forms of arsenic were only detected in a small number of urine samples in NHANES, arsenocholine (1.8%) and trimethylarsine oxide (0.3%). Although it is worth noting that arsenobetaine is the predominant urinary metabolite of arsenocholine, at least in rats, mice and rabbits (Marafante *et al.* 1984). In any event, urinary arsenobetaine due to the ingestion of arsenocholine would still ultimately be attributed to consumption of organic arsenic. Urine can also contain thioarsenicals which were not analyzed in this study.

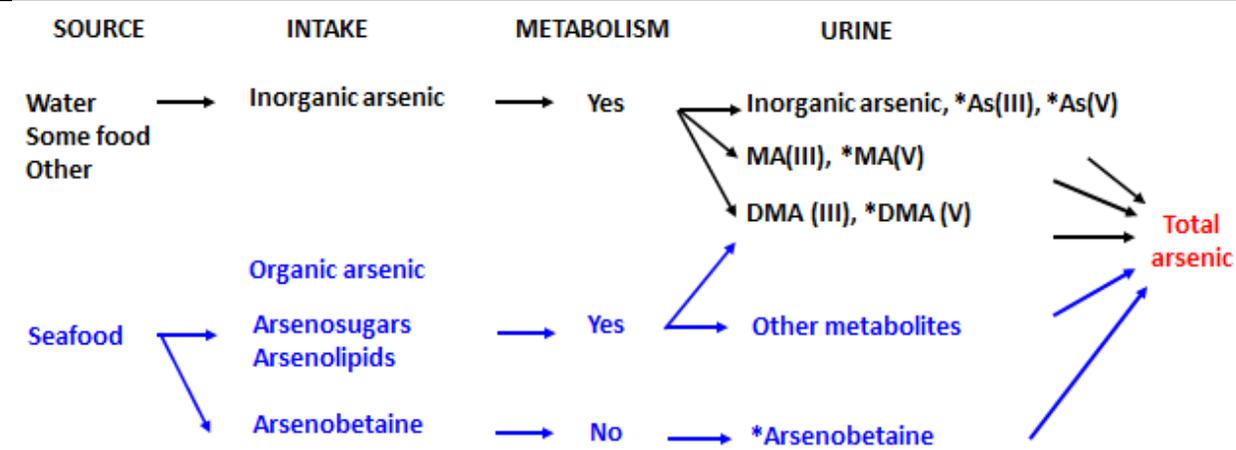
Three general approaches have been proposed to account for organic arsenic of seafood origin in NHANES (1) restrict the analysis to participants not likely to have consumed seafood close to the time of sample collection by restricting the sample to participants with very low or

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undetectable arsenobetaine (Navas-Acien *et al.* 2008, 2009a), (2) statistically adjust for urinary levels of arsenobetaine or blood mercury as markers for seafood consumption (Navas-Acien *et al.* 2008, 2009a), and (3) subtract from the total urinary arsenic measurement any organic arsenicals that were detected in NHANES participants, i.e., arsenobetaine and arsenocholine (if above the detection limit)¹¹(Steinmaus *et al.* 2009a). These three general strategies lead to different conclusions on the association between inorganic arsenic and diabetes in NHANES.

Figure 6. Accounting for organic arsenic of seafood origin in NHANES



Modified from Figure 1 of Navas-Acien *et al.* (2009a).

*Arsenic species measured in NHANES (Caldwell *et al.* 2009). Two other organic forms of arsenic considered to be minor contributors to arsenic in seafood were also measured in NHANES but only detected in a small number of urine samples, arsenocholine (1.8%) and trimethylarsine oxide (0.3%). The predominant urinary metabolite of arsenocholine in rats, mice and rabbits is arsenobetaine (Marafante *et al.* 1984)

In the initial 2008 publication by Navas-Acien *et al.*, the authors controlled for seafood intake by restricting the analysis to participants who did not report seafood intake in the 24-hour period prior to sample collection and adjusting total urine arsenic for objective measures of seafood intake (urinary arsenobetaine and blood mercury). The result after correcting for other factors (e.g., age, sex, body mass index, etc.) was a 3.58-fold increase (95% CI, 1.18-10.83) in diabetes at the 80th (16.5 µg/L) versus the 20th (3.0 µg/L) percentiles of total urinary arsenic. Using the same NHANES 2003-2004 data as Navas-Acien *et al.*(2008), but taking the approach of subtracting arsenobetaine and arsenocholine from total arsenic, Steinmaus *et al.*(2009a) found no association between arsenic and diabetes when comparing participants ≥ 80th vs. ≤ 20th percentiles of total urinary arsenic, OR 0.88 (95% CI 0.39-1.97). One criticism of the approach used by Steinmaus *et al.*(2009a) is that subtracting arsenobetaine and arsenocholine from the total will not remove other organic forms of arsenic not specifically measured in NHANES but included in the measure of total urinary arsenic (Navas-Acien *et al.* 2009a). In addition, DMA is the main metabolite of arsenosugars and arsenolipids and the approach used by Steinmaus *et al.*(2009a) would not account for DMA that may be of seafood origin. In addition, the portion of

¹¹Steinmaus *et al.* (2009a) did not consider trimethylarsine oxide because it was only detected in 0.3% NHANES participants.

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DMA in NHANES due to the metabolism of inorganic arsenic or arsenosugars/arsenolipids from seafood likely differs at lower levels of total urinary arsenic compared to higher levels. In NHANES 2003–2004, DMA was the major contributor to total urinary arsenic at lower levels (<20 µg/L), with a median contribution of 53.8%. At higher levels of total urinary arsenic (≥ 50 µg/L) arsenobetaine was the major form with a median contribution of 62.7% to the total (Caldwell *et al.* 2009). In participants where arsenobetaine is the major contributor to total urinary arsenic, DMA in urine was also likely to reflect exposure to organic arsenicals in seafood, since DMA is a metabolite of arsenosugars and arsenolipids that co-occur with arsenobetaine in seafood. In NHANES 2003-2004, the correlation coefficient between arsenobetaine and DMA was 0.48 (Dr. Ana Navas-Acien, personal communication April 2010). These complexities can be avoided by controlling for seafood ingestion although there may be a loss of statistical power because the number of NHANES participants included in the analysis will be reduced.

Navas-Acien *et al.*, (2009a) extended the original NHANES 2003-2004 analysis to include data from NHANES 2003-2006 but this time accounted for organic arsenic of seafood origin by restricted the analysis to participants with undetectable arsenobetaine (≤0.4 µg/L; n = 381, 62 with diabetes). After adjustment for sociodemographic and diabetes risk factors, the OR for diabetes was 2.60 (95% CI 1.12– 6.03) comparing participants at the 80th versus the 20th percentiles of total urine arsenic (7.4 vs. 1.6 µg/L) and 4.26 (95% CI 0.83-21.8) in participants ≥80th vs. ≤20th percentile of total urine arsenic. The impact of inadequately accounting for seafood-derived arsenicals appears to be more of a factor at higher total urinary arsenic levels where organic arsenic from seafood (arsenobetaine) is the predominant contributor as noted above (Caldwell *et al.* 2009). Navas-Acien *et al.* (2009a) found considerable potential for exposure misclassification when inorganic arsenic was estimated by total urine arsenic minus arsenobetaine and arsenocholine compared to using total urine arsenic in participants with undetectable arsenobetaine. Total arsenic minus arsenobetaine and arsenocholine at the 20th, 50th, 80th, 90th, and 99th percentiles was 2.7, 11.9, 18.4, and 73.6 µg/L, respectively, while the distribution of total urine arsenic among participants with undetectable urine arsenobetaine at the same percentiles was 1.9, 3.9, 7.7, 11.8, and 30.4 µg/L, respectively.

Adjusting Urinary Levels of Arsenic for Creatinine Concentration

Typically, epidemiology studies that quantify exposure based on spot urine measures for arsenic or other non-persistent chemicals include adjustments for urine creatinine to account for variation in urine dilution. This may be accomplished through normalizing arsenic levels for creatinine as the exposure metric (i.e., $\mu\text{g As/g urinary creatinine}$) or adjusting by using urine arsenic as the measure of exposure ($\mu\text{g As/L urine}$), but then including creatinine as a separate independent variable in the multiple regression analyses. Of the two approaches, the latter approach is recommended (Barr *et al.* 2005) because urinary creatinine concentrations are influenced by age, sex, health status, race/ethnicity, body mass index, fat-free mass, and time of day of collection and can therefore vary widely across individuals (Barr *et al.* 2005; Boeniger *et al.* 1993; Mahalingaiah *et al.* 2008). For example, the range of urinary creatinine concentrations in NHANES 2007-2008 was over 200-fold (3–724 mg/dL) (CDC 2009), much greater than the range of urine volume excreted in a day (Boeniger *et al.* 1993). Inclusion of creatinine in the multiple regression model allows urinary As concentrations to be adjusted for creatinine while permitting the evaluation of other variables independent of the effects of creatinine concentration.

This issue of creatinine adjustment has been a topic of some debate with respect to the cross-sectional studies of diabetes, especially for findings based on NHANES data (Longnecker 2009; Navas-Acien *et al.* 2009a; Steinmaus *et al.* 2009b). Adjustment for urinary creatinine has the effect of making the regression beta between As and type 2 diabetes more positive. This pattern is observed in Chen *et al.* (2010) and Steinmaus *et al.* (2009b). In both studies, the OR value was numerically larger (but not necessarily statistically significant) when urinary creatinine concentration was included as a variable in the multiple regression model (Table 5).¹² Less clear is whether creatinine adjustment provides a better estimate of truth of the association.

The decision on how, or whether, to adjust for urinary creatinine concentration is more complicated when the health effect under investigation can impact creatinine levels, as is the case with diabetes (Greenland 2003). People with type 2 diabetes tend to have lower urinary concentrations of creatinine. In the Third NHANES (1988-1994) creatinine levels in the urine of people with diabetes 30–39 years of age were 40.6 mg/dL lower than those without diabetes in this age group ($p=0.011$) (Barr *et al.* 2005).

People with diabetes have lower urinary creatinine concentrations for two main reasons. First, the lower muscle mass in patients with type 2 diabetes (Park *et al.* 2009) results in less creatinine in urine. If this were the only effect of type 2 diabetes on urinary creatinine then adjustment for creatinine would produce a positive bias of an association between total arsenic and type 2 diabetes in cross-sectional studies because adjusting urine As by creatinine would result in a higher numerical indicator of As exposure in people with diabetes compared to people without diabetes. For example, creatinine adjustment of a measurement of 10 $\mu\text{g As/L}$

¹² A similar comparison cannot be made for Navas-Acien *et al.* (2009a) because the authors always adjusted for creatinine.

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urine would result in a 2-fold higher value in diabetics if they had half the amount of urine creatinine as non-diabetics, e.g., 20 $\mu\text{g As/g creatinine}$ based on 0.5 g creatinine/L urine versus 10 $\mu\text{g As/g creatinine}$ based on 1 g creatinine/L urine. The magnitude of the effect would depend on how long subjects had had type 2 diabetes, the severity of their disease, etc.

Second, the hyperfiltration (increased glomerular filtration rate) associated with diabetes causes urine to be more dilute, which is reflected in a lower urinary creatinine concentration (Jerums *et al.* 2010). The dilute urine due to hyperfiltration could cause a lower urinary arsenic concentration to decrease, though the extent of the decrease would depend on whether contaminated water or other fluids were consumed in response to higher fluid requirements. If the replacement fluid was lower in arsenic than the tap water, then there would be a decrease in urine arsenic concentration due to hyperfiltration. In that case, failure to adjust cross-sectional analyses for urinary dilution could cause the appearance of an inverse association of total arsenic with type 2 diabetes, i.e., reduced diabetes prevalence with increasing urine As, when in fact no causal association exists. Furthermore, if a real, positive association existed between type 2 diabetes and arsenic, failure to adjust could make an association appear smaller than it really is. In either case, the result would be a bias away from identifying a positive association between arsenic and type 2 diabetes. Speciation analysis in cells or tissues is needed to properly estimate the internal dose.

However, not all diabetics have lower urinary creatinine levels and this association appears stronger in certain age and racial/ethnic populations. In the Third NHANES (1988-1994) urinary creatinine levels for people with diabetes 30–39 years of age were 40.6 mg/dl lower than those without diabetes in this age group ($p=0.011$) (Barr *et al.* 2005). However, when looked at within racial/ethnic categories, urine creatinine levels were only significantly lower in non-Hispanic black participants and not other racial/ethnic categories.

The situation is further complicated because arsenic exposure has also been positively associated with urine creatinine in people living in an arsenic endemic area of Bangladesh (Nermell *et al.* 2008) or participating in the HEALS study described above (personal communication with Dr. Ahsan Habibul, July 17, 2010). It is unclear the extent to which these findings are generalizable to other regions given that Bangladeshi people are more prone to malnourishment than other populations, like Americans. People who are malnourished tend to have less muscle mass and, consequently, lower urinary creatinine levels. Nermell *et al.* (2008) reported urinary creatinine concentrations of 0.55 g/L in adolescents and adults from Matlab, Bangladesh where ~30% of adults could be defined as underweight (BMI < 18.5) compared to typical creatinine levels in the US, Europe, and Japan of 1 g/L for women and 1.5 g/L for men. The result is that creatinine adjusted urinary As concentrations in the Bangladeshi would be higher compared to other populations with the same numerical values for urine As. For example, 10 $\mu\text{g As/L urine}$ would equal 18 $\mu\text{g As/g creatinine}$ in the Matlab population compared to an estimate of 10 $\mu\text{g As/g creatinine}$ in US women.¹³ Nermell *et al.* (2008)

¹³ Based on the average 0.55 g creatinine/L urine reported by Nermell *et al.* (2008) in residents of Matlab, Bangladesh and an average 1 g creatinine/L urine in women living in the U.S. 10 $\mu\text{g As/L urine}$ = 10 $\mu\text{g As}/0.55 \text{ g}$

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reported that urinary creatinine was positively associated with arsenic metabolites in urine ($r = 0.19$ to 0.26 , depending on age), and the correlation was mainly with the DMA metabolite. A positive correlation between total urinary arsenic and urine creatinine, $r = 0.43$, was also observed in the HEALS study (personal communication with Dr. Ahsan Habibul, July 17, 2010). These correlations make sense given that both creatinine and DMA are products of S-adenosylmethionine (SAM) dependent methylation reactions. The predicted impact of this on indicators of arsenic exposure is that creatinine adjustment of urine As will lead to increasing underestimation of the “true” exposure with increasing exposure (Nermell *et al.* 2008). It is unclear how generalizable a positive association between arsenic and creatinine is, even in Bangladesh. Nabi *et al.* (2005) reported that serum creatinine levels in a smaller study of 115 arsenic-affected patients (based on the appearance of skin lesions and high drinking water and urine levels of arsenic) were significantly lower compared to a reference group of 120 people (0.97 versus 1.15 mg/dL, $p=0.007$).

If arsenic exposure and diabetes are associated with altered creatinine levels then urine creatinine may function as a “collider” variable (diabetes \rightarrow urine creatinine \leftarrow arsenic) as opposed to a classic confounder (diabetes \leftarrow urine creatinine \rightarrow arsenic) (Greenland 2003). Thus, creatinine level fails the test for a confounder because it is not an independent risk factor for diabetes, but rather a consequence of it. Given this, adjustment for creatinine may increase bias rather than reducing it. However, given the issues discussed above it may not be possible to fully understand the potential bias with respect to studies of arsenic and diabetes.

Alternatively, specific gravity (SG) has been suggested as an alternative way to normalize urine As for differences in urine dilution because it appears to be less affected than creatinine by age, gender, and body size (Mahalingaiah *et al.* 2008; Nermell *et al.* 2008). It is unclear whether this is a suitable strategy to look at diabetes and may depend on the degree of glucose present in the urine. It is not possible to address this issue in NHANES data because specific gravity was not measured.

creatinine per liter urine = $18 \mu\text{g As/g creatinine}$ in the Bangladeshi and $10 \mu\text{g As/1 g creatinine per liter urine} = 10 \mu\text{g As/g creatinine}$ in women living in the U.S.

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Table 5. Impact on ORs by including urine creatinine in the multiple regression model

	Bangladesh, HEAL study Chen et al. (2010)		US, NHANES 2003-06		
	$\mu\text{g As/L urine}$	OR for diabetes prevalence	As exposure category	OR for diabetes prevalence	OR for diabetes prevalence
Findings with and without model adjustment for urine creatinine	Q1: 0-8.0	1.0	80 th vs. 20 th percentile*	unadjusted: NR	NA
	Q2: 8.1-41.0	unadjusted: 1.29 (0.87-1.91) adjusted: 1.44 (0.97-2.17)		adjusted: 2.60 (1.12-6.03)	
	Q3: 41.2-91.7	unadjusted: 1.05 (0.69-1.59)	$\geq 80^{\text{th}}$ vs. $\leq 20^{\text{th}}$ percentile*	unadjusted: NR	unadjusted: 1.03 (0.38-2.80) adjusted as continuous variable: 2.32 (0.51-10.5)** adjusted as categorical variable: 1.19 (0.10-14.0)
		adjusted: 1.20 (0.77-1.85)		adjusted: 4.26 (0.83-21.8)	
	Q4: 91.8-176.1	unadjusted: 0.94 (0.61-1.44) adjusted: 1.16 (0.73-1.85)			
Q5: 176.2-864	unadjusted: 0.93 (0.59-1.45) adjusted: 1.22 (0.73-2.03)				
BMI of study population	<22 in ~80% of participants		≥ 25 in ~68% of participants [from NHANES 2003-04 as presented in Navas-Acien et al. (2009a)]		

* Analysis conducted in participants with undetectable arsenobetaine to control for inorganic arsenic from seafood consumption

**Note from Steinmaus et al. (2009b): "With NHANES sampling weights, further adjustment (for education, serum cotinine, and hypertension medication), and using slightly different quintile cut-off points, Navas-Acien et al. reported this odds ratio as 4.26 (0.83–21.8).

NR = not reported

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Health Impacts of Arsenic in People with Diabetes

A study by Chiou et al.(2005) suggests that health impacts of arsenic may be exacerbated in people with diabetes living in arsenic-endemic regions. Chiou et al.(2005) evaluated the medical records of 28,499 people living in Taiwan based on residential records and information contained in databases of arsenic water contamination. The prevalence of microvascular and neurological disease increased significantly with arsenic exposure, especially at higher water arsenic concentrations ($\geq 60 \mu\text{g/L}$), and this association was stronger in people with diabetes compared to those without the condition (Table 6). If this finding is generalizable, it suggest that aspects of arsenic toxicity might be exacerbated in populations with much higher diabetes incidence than Bangladesh. The greater impact of arsenic in diabetics was also looked at in Wistar rats that were made diabetic by alloxan treatment and then treated with 40 mg/kg arsenic trioxide, As_2O_3 , by injection on alternating days for one month (Singh and Rana 2009).This arsenic dose level was high enough to cause weight loss in animals, an indication of general toxicity. Based on assessment of liver function and liver arsenic levels the authors concluded that the pharmacokinetics and pharmacodynamics of arsenic may differ in diabetic animals. Liver arsenic concentrations were significantly less in diabetic rats compared to the non-diabetic rats (305 $\mu\text{g/g}$ versus 853 $\mu\text{g/g}$).

Table 6. Stronger associations between water concentrations of arsenic and prevalence of microvascular, neurological, and renal disease in diabetes compared to non-diabetics (Chiou *et al.* 2005)

Disease	Diabetes Status	Water Arsenic Concentration ($\mu\text{g/L}$)			
		< 10	10-29	30-59	≥ 60
microvascular disease (% prevalence)	diabetics (n = 2399)	16.41	15.85	21.69	28.31
	non-diabetics (n=26,100)	7.51	6.59	8.02	11.82
	p-value for trend in both groups < 0.005				
neurological disease (% prevalence)	diabetics (n = 2399)	8.89	9.53	14.94	21.36
	[OR (95% CI)]	[1.00]	[1.08 (0.73-1.6)]	[1.80 (1.32-2.46)]	[2.78 (2.01-3.85)]
	non-diabetics (n = 26,100)	6.97	5.94	7.65	11.19
	[OR (95% CI)]	[1.00]	[0.84 (0.73-0.97)]	[1.10 (0.98-1.25)]	[1.68 (1.49-1.89)]
renal disease (% prevalence)	diabetics (n = 2399)	1.92	1.34	2.54	3.91
	[OR (95% CI)]	[1.00]	[0.69 (0.27-1.81)]	[1.33 (0.67-2.63)]	[2.08 (1.05-4.11)]
	non-diabetics (n = 26,100)	0.55	0.64	0.42	0.71
	[OR (95% CI)]	[1.00]	[1.17 (0.75-1.83)]	[0.78 (0.48-1.24)]	[1.30 (0.85-2.00)]

1.4 Experimental Animal Studies

As noted earlier, risk characterizations of arsenic often rely on epidemiological studies. Laboratory animals are generally considered less sensitive than humans to arsenic toxicity, mainly due to species differences in arsenic metabolism and other aspects of toxicokinetics. Thus animal studies have generally been used in a more supportive role in arsenic risk evaluations and to provide mechanistic foundations for understanding arsenic toxicity (ATSDR 2007; California EPA 2004; EFSA 2009).

As with the human literature, more animal studies have assessed endpoints relevant to diabetes rather than obesity, i.e., glucose levels, glucose tolerance, and pancreatic function. Overall, the laboratory animal studies have considerable variation in experimental design which can make them difficult to interpret for consistency of specific findings, e.g., direction of effect on basal glucose levels. Appendix Table B summarizes ~25 animal studies published between 1979 and 2009 that were identified for this review, the majority of which were conducted in rats or mice. Most of the studies treated animals with sodium arsenite, As(III), or arsenic trioxide, As(III) oxide, but studies have also been published for arsenic pentoxide, As(V) oxide (Aguilar *et al.* 1997), monomethylarsonic acid (MA) (Arnold *et al.* 2003), sodium arsenate, As(V) (Hill *et al.* 2009), and methylarsine oxide, MAs(III) oxide (Paul *et al.* 2008). There is also considerable variation in the duration of treatment (one day to two years), routes of administration [drinking water, intraperitoneal (ip) or subcutaneous (sc) injection, gavage, diet, or capsule], and dose levels used in the studies.

Despite these variations in experimental design, alterations (either decrease or increase) in blood/urinary glucose or impaired glucose tolerance are common. In many cases the studies only assessed glucose homeostasis and did not attempt to identify a mechanistic basis for the findings; however some identified pancreatic effects following arsenic treatment (Arnold *et al.* 2003; Boquist *et al.* 1988; Izquierdo-Vega *et al.* 2006; Mukherjee *et al.* 2006; Yen *et al.* 2007) or co-treated groups of animals with other compounds to determine whether the effects of arsenic can be attenuated (Hill *et al.* 2009; Mukherjee *et al.* 2006; Pal and Chatterjee 2004a, b, 2005). These studies suggest that animal models can be useful to understand effects of arsenic on glycemic control. However, tissue analysis of arsenic in studies conducted by Paul *et al.* (2008; 2007b) also support the general notion that rodent are less susceptible to arsenic-induced toxicity than humans based on differences in metabolism and elimination. More specifically mice seem less susceptible because of faster metabolism and clearance of arsenic (i.e., lower internal dose). Rats, unlike mice or humans, sequester arsenic (specifically DMA) in erythrocytes. It is unclear how this binding affects susceptibility to inorganic arsenic exposure and rats are generally not recommended as a model for arsenic toxicity. It is essential that animal studies examining the diabetogenic effects of arsenic provide information about tissue concentrations of total arsenic and arsenic species as a point of reference for comparison with human exposures. Unfortunately, data on As concentration/speciation in human tissues are very scarce or not existent (for chronic exposures). Nevertheless, the animal studies can be useful for considering the biological plausibility of arsenic as a diabetogenic agent but they will have limited utility as a basis for understanding potential effects in humans, especially at low-

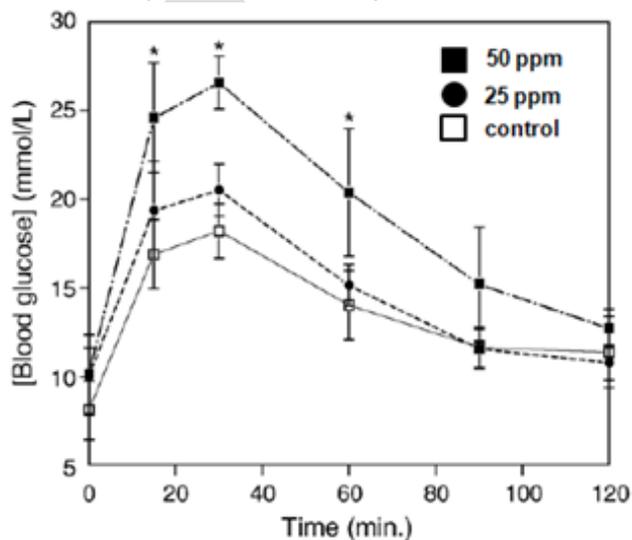
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to-moderate arsenic exposures, without additional internal dosimetry, human biomonitoring, and/or dose-response modeling efforts. Several studies are described below to illustrate the nature of the reported findings.

Paul et al.(2007b) reported impaired glucose tolerance (but no significant difference in fasting glucose) in weanling C57BL/6 mice fed a grain-based laboratory diet and treated with 50 ppm sodium arsenite (As(III)), in drinking water for 8 weeks (Figure 7). The C57BL/6 mouse strain was selected based on a low reported baseline incidence of type 2 diabetes and high susceptibility for developing diet-induced type 2 diabetes (Petro *et al.* 2004; Surwit *et al.* 1995; Surwit *et al.* 1988). Statistically significant changes in glucose tolerance were not observed at a lower dose of 25 ppm. The C57BL/6 mice treated with As(III) were considered to be less susceptible than humans to arsenic-induced toxicity based on more efficient metabolism and clearance of inorganic arsenic. This conclusion was based on observing similar liver concentrations of total arsenic in the treated mice compared with liver samples collected from residents of an arsenic-endemic area of Bangladesh who developed hepatomegaly associated with consuming lower drinking water levels of arsenic (0.22–2 ppm; Table 7).

Figure 7. Impaired glucose tolerance following treatment with 50 ppm sodium arsenite, As(III), in C57BL/6 mice (Paul et al. 2007b)



Blood glucose before and during ip glucose tolerance test. From Figure 3 in Paul et al.(2007b), *p<0.05

In a separate study, Paul et al.(2008) did not observe any effects on fasting blood glucose or glucose tolerance in C57BL/6 mice treated with a major toxic metabolite of inorganic arsenic, methylarsonous acid, MAs(III), at 0.1, 1, 2.5, or 5 ppm in drinking water.

Table 7. Higher liver concentrations of total arsenic in C57BL/6 mice compared to humans after similar drinking water exposures (Paul et al. 2007b)

Species	Drinking water level (ppm)	Liver concentration (total As, µg/kg intact liver)
human (Bangladesh)	0.22–2	~100–1200*
mouse (C57BL/6)	1–10	11–155
	25–50	423–1165

From Paul et al.(2007b); *Estimated from dry-weight values from Mazumder et al., 2005 as cited in Paul et al.(2007b)

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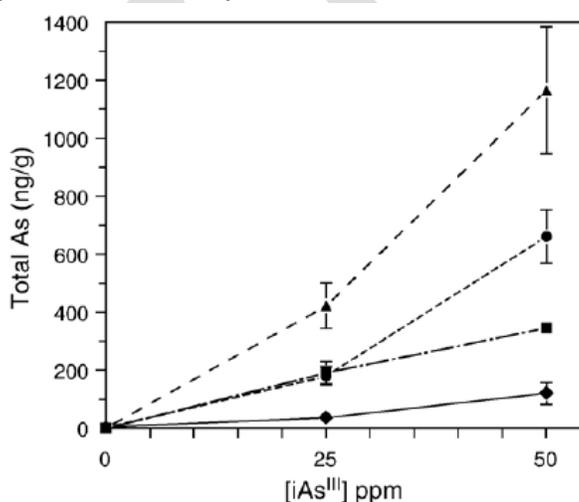
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Paul et al. (2008; 2007b) also measured arsenic in tissues relevant for type 2 diabetes (liver, pancreas, skeletal muscle and adipose). Arsenic was detected in all these tissues in every As(III) and MAs(III) treatment groups and was present mostly as DMA and MA¹⁴. A notable finding from Paul et al.(2007b) is that liver and skeletal muscle concentrations of As(III) were disproportionately greater following treatment with 50 ppm compared to 25 ppm (Figure 8) (Paul *et al.* 2007b). This may be partly due to a decrease in water intake that was observed in the 50 ppm treatment group which would result in less urine production and possibly less efficient excretion of inorganic arsenic metabolites compared to the 25 ppm group.

Tissue level analysis may also explain the absence of an effect of MAs(III) in the glucose tolerance test despite *in vitro* indications of increased potency compared to As(III) in inhibiting insulin-stimulated glucose uptake in 3T3-L1 adipocytes ($\geq 0.5 \mu\text{M}$ MA(III) versus $\geq 5 \mu\text{M}$ As(III) (Paul *et al.* 2007a). Tissue levels of total arsenic were disproportionately lower in animals treated with the highest dose of MAs(III) tested, 5 ppm, compared to animals treated with 25 ppm As(III) in Paul et al.(2008). The liver concentrations of in the MAs(III)-treated mice were more than 25-times lower than the liver levels reported following treatment with As(III), suggesting that, at these concentrations, MAs(III) is metabolized by mice much faster than As(III).

New unpublished data from Paul et al. (2010) extended these findings to look at potential interactions between treatment with As(III) and obesity/adiposity by comparing responses in C57BL/6 mice fed a high fat (HF) diet or low fat (LF) diet. All animals fed a HF diet showed the expected increase in body weight and fat mass compared to LF diet counterparts, but the magnitude of this effect was less in mice drinking water with 50 ppm As(III). Several other HF

Figure 8. Liver, skeletal muscle, pancreatic, and adipose tissue concentrations of total arsenic in C57BL/6 mice treated with 25 or 50 ppm As(III) in drinking water (Paul et al. 2007b)



Dose-dependent increases in the total speciated As ($i\text{As}^{\text{V}} + \text{MAs}^{\text{V}} + \text{DMA}^{\text{V}}$) levels in adipose tissue (◆), pancreas (■), skeletal muscle (●), and liver (▲). From Figure 4 in Paul et al.(2007b)

¹⁴ The relative ranking of tissue concentrations following treatment with As(III) was liver > skeletal muscle > pancreas > adipose tissue. Arsenic concentrations in liver and skeletal muscle levels were more similar following treatment with MA(III) (liver \approx skeletal muscle > pancreas > adipose)¹⁴(Paul *et al.* 2008). Arsenic was also detected in tissues collected from control animals, which may be explained by the presence of arsenic in the rodent chow. Concentrations of total arsenic in the diet used in these studies (Lab Diet 5058, Nutrition International) ranged from 20 to 66 ppb total arsenic, of which 70 to 80% was inorganic arsenic (Paul *et al.* 2008).

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diet-induced impairments related to insulin or glucose were also less pronounced in this treatment group, i.e., HOMA-IR, insulin sensitivity, and fasting blood glucose. However, the authors found that glucose intolerance remains pronounced in mice fed HF diet and exposed to 25 and 50 ppm As(III) compared in spite of only moderate adiposity and improved insulin resistance. Overall, the findings were interpreted as suggesting that the diabetogenic effects of As(III) differ from type-2 diabetes typically associated with diet-induced obesity.

Impaired glucose and insulin profiles have also been observed in other studies with mice (Boquist *et al.* 1988; Hill *et al.* 2009; Yen *et al.* 2007) or rats (Cobo and Castineira 1997; Ghafghazi *et al.* 1980; Izquierdo-Vega *et al.* 2006; Singh and Rana 2009; Wang *et al.* 2009). A study by Izquierdo-Vega *et al.* (2006) assessed a number of endpoints relevant to glycemic control and the pancreas in male Wistar rats treated with 1.7 mg/kg sodium arsenite, As(III), by gavage twice daily for 90 days. The As(III)-treated rats developed hyperglycemia, hyperinsulinemia, insulin resistance, and had a ~10% decrease in serum glucagon (Table 8). In the pancreas there was less insulin labeling in β cells and indications of stress and oxidative damage based on higher glutathione levels, less thioredoxin reductase activity (TrxR), and more lipoperoxidation based on higher levels of thiobarbituric acid reactive substances (TBARS).

Hill *et al.* (2009) used LM/Bc/Fnn inbred mice¹⁵ to evaluate the role of glucose intolerance in the development of arsenate-induced neural tube defects. This research question was of interest because maternal hyperglycemia is a well-established teratogen in both humans and laboratory animals. Pre-gestational diabetes in women is associated with a 2- to 10-fold increase in the risk for congenital neural tube defects (McLeod and Ray 2002). Pregnant mice were treated by ip injection with 9.6 mg/kg bw sodium arsenate, As(V), on GD7.5 and 8.5, a treatment regime known to cause exencephaly in nearly all offspring¹⁶. Compared to water-treated control animals, the dams had impaired glucose tolerance test with significantly higher glucose levels at 15, 30, 60, 90, and 120 minutes after glucose challenge (insulin response was unaffected), and significantly higher levels of fasting glucose (100 versus 71 mg/dl), non-fasted glucose (138 versus 113 mg/dl), fasting insulin (15.7 versus 13.9 μ U/ml), and insulin resistance (HOMA-IR of 3.9 versus 2.5). These latter findings are consistent with those reported by Izquierdo-Vega *et al.* (2006) in rats following oral treatment with sodium arsenite, As (III), described above (Table 8).

Table 8. Effects of twice daily gavage treatment with 1.7 mg/kg sodium arsenite, As(III), on glycemic control in Wistar rats (Izquierdo-Vega *et al.* 2006)

parameter	control	iAs ³⁺
glucose (mmol/L)	5.42	9.99*
insulin (ng/ml)	0.75	1.93*
glucose:insulin ratio	10.67	4.82*
HOMA-IR	3.34	13.17*
glucagon (pg/mL)	~2.7	~2.5*

From Izquierdo-Vega *et al.* (2006), *p \leq 0.01

¹⁵ LM/Bc/Finn inbred mice have been maintained in the Finnell laboratory for several decades due to their low incidence of spontaneous malformations and resistance to most teratogens investigated in the lab.

¹⁶ The authors used ip injection as the route of administration because oral maternal treatment with arsenic only causes a modest increase in exencephaly: (water, 0.0%; As 4.8 mg/kg, 0.8%; As 9.6 mg/kg, 3.7%; As 14.4 mg/kg, 8.6%) [Hill *et al.* (2008) as cited in Hill *et al.* (2009)], presumably because the ip injection results in higher exposure to the fetus and possible differences in metabolism of iAs.

1.4.1 "Rescue" Studies

Results from several studies suggest that co-treatment with methyl donors or antioxidants (e.g., folic acid, vitamin B12, methionine, N-acetyl cysteine) may attenuate the effects of arsenic toxicity (Hill *et al.* 2009; Mukherjee *et al.* 2006; Pal and Chatterjee 2004a, b, 2005). Methyl donor compounds have been used as co-treatments because the methylation of inorganic arsenic to MA and DMA facilitates clearance of arsenic from the body. Antioxidants have been used because oxidative stress is considered a major pathway in arsenic-induced toxicity.

Mukherjee *et al.* (2006) reported that the methyl donors folic acid and vitamin B₁₂ reduced the degree of arsenic-induced pancreatic toxicity. Pancreatic islet cell counts were reduced to 35 – 50% of control values in male albino rats following oral administration of 3 mg/kg bw/day arsenic trioxide for 30 days compared to control animals. This dose of arsenic is within the range of the LD50 value for humans (1-4 mg/kg) and less than 10% of the LD50 value for rats (40 mg/kg). The effect on islet cells was reduced when animals were co-treated with 36 µg/kg bw/day folic acid (from 67% to 87% of control values) and to an even greater degree in animals treated with this dose of folic acid and 0.63 µg/kg bw/day vitamin B₁₂ (from 87% to 94% of control values). A similar restorative pattern was reported for certain measures of cellular oxidative damage or inflammation in the pancreatic tissue of arsenic-treated rats (NO, MDA, OH⁻, SOD, CAT, GSH, TNF-α, and IL-6). However, effects of the methyl donor supplementation on arsenic metabolism and disposition were not examined.

In the Hill *et al.* (2009) study described above, the incidence of neural tube defects was 100% in animals treated with 9.6 mg/kg bw sodium arsenate, As(V), on GD7.5 and 8.5. This 100% incidence of arsenate-induced neural tube defects was significantly reduced in animals co-treated with several "rescue" agents: N-acetyl cysteine (NAC)¹⁷ (38.2%); the insulin pellet LinBit (46.4%); methionine, a methyl donor (67.9%); N-tert-butyl-α-phenylnitron (PBN), a compound that traps and stabilizes oxidative radicals (74.1%); and sodium selenate (SS)¹⁸, the active center of antioxidant seleno-enzymes such as glutathione peroxidase (76.6%). Despite the efficient "rescue" by NAC (from 100% to 38.2% neural tube defects) this treatment did not significantly alter AS(V)-induced elevations in fasting plasma glucose or insulin (the impacts of methionine, PBN, and SS on fasting plasma glucose and insulin were not evaluated).

Pal *et al.* (2004a, b, 2005) also reported a reversal of certain aspects of arsenic toxicity in animals co-treated with the methionine (methyl donor), NAC (antioxidant), and melatonin (free radical scavenger). In all studies, male Wistar rats were ip injected with a high dose of sodium arsenite, As(III), for 21 to 30 days (5.55 mg/kg bw/day, ~35% of the LD50 dose). Towards the end of this treatment period some of the arsenic-treated animals were then co-treated with 0.8% methionine in the diet (Pal and Chatterjee 2004a), 163.2 mg/kg/day NAC by oral

¹⁷ NAC acts as an antioxidant increasing GSH concentration in tissues, but it also binds AsIII, changing its bioavailability and metabolism. NAC and methionine are sulphur-containing molecules that can chelate As.

¹⁸ Selenate can be reduced in the body to selenite, which is a potent inhibitor of inorganic arsenic methylation. This effect of Se may be more important in this case than its antioxidant properties

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treatment (Pal and Chatterjee 2004b), or 10 mg/kg/day melatonin by ip injection (Pal and Chatterjee 2005). Fasting plasma glucose levels were significantly lower (hypoglycemia) in arsenic-treated animals in all the studies (46.6 – 68.3 mg/100 ml versus 84.7 – 98.3 mg/100 ml in pair-fed control animals) and this effect was attenuated in animals co-treated with sodium arsenite, As(III) and methionine (81 mg/100 ml), NAC (93.5 mg/100 ml), or melatonin (69 mg/100 ml).

1.5 *In vitro*/Mechanistic Studies

To be discussed at the workshop

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Appendix Tables
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1.6 Appendix Tables

Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For			
cross-sectional Pakistan (Afridi <i>et al.</i> 2008) environmental, low to moderate	434 men living in Hyperabad, Pakistan (31-60 years of age)	total: 196 (45.2%)/238	diabetes (self-report)	scalp hair, µg/g	smokers (mean hair, µg/g)	2.89 diabetics vs. 1.47 non-diabetics (t-test, p <0.05)	none		
		209 smokers: 86 (41.1%) cases/123		non-smokers (mean hair, µg/g)	1.67 diabetics vs. 0.98 non-diabetics (t-test, p <0.05)				
		225 non-smokers: 110 (48.9%) cases/115		blood, µg/l	smokers (mean blood, µg/l)			3.78 diabetics vs. 2.48 non-diabetics (t-test, p <0.05)	
				non-smokers (mean blood, µg/l)	2.59 diabetics vs. 1.95 non-diabetics (t-test, p <0.05)				
			urine, µg/l	smokers (mean urine, µg/l)	7.27 diabetics vs. 5.41 non-diabetics (t-test, p <0.05)				
				smokers (mean urine, µg/l)	5.59 diabetics vs. 4.7 non-diabetics (t-test, p <0.05)				
retrospective cohort Italy (Bartoli <i>et al.</i> 1998) occupational	Cause of death in 3,180 male art glass workers employed in 17 facilities in Tuscany, IT (488 total deaths; <40-65+ years of age)	488 deaths 3 diabetes deaths (0.6%)/ 485 other	diabetes (death certificate)	job title	glass workers vs. general population for Tuscany	SMR=0.34 (90% CI: 0.09-0.88)	age, gender, calendar year		
cross-sectional Bangladesh (Chen <i>et al.</i> 2010) environmental, low to moderate & HAA	Baseline data from 11,319 participants in the Health Effects of As Longitudinal Study (HEALS) 2000-2002 (43% male; ≥18 years of age)	241 cases/ 10,837 non-cases	diabetes (self-reported physician diagnosis prior to baseline; participants diagnosed after baseline exam were excluded)	time weighted water As (µg/L)	Q1: 0-8.0 µg/L water (referent)	OR=1.00	age, gender, BMI, smoking status, and educational attainment (similar results obtained when model only adjusted for age, gender, and		
					n=2258, 52 diabetes (2.3%)/2206 non-diabetics			Q2: 8.1-41.0	1.35 (0.90-2.02)
					Q3: 41.2-91.7 n=2151, 49 diabetes (2.3%)/2102 non-diabetics			1.24 (0.82-1.87)	

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For
					Q4: 91.8-176.1 0.96 (0.62-1.49)	BMI)
					Q5: 176.2-864 n=2184, 44 diabetes (2.0%)/2140 non- diabetics	1.11 (0.73-1.69)
				urine total As (µg/L)	Q1: 1-36 µg/L urine n=2210, 50 diabetes (2.3%)/2160 non- diabetics	OR=1.00
					Q2: 37-66 1.29 (0.87-1.91)	
					Q3: 67-114 n=2172, 46 diabetes (2.1%)/2126 non- diabetics	1.05 (0.69-1.59)
					Q4: 115-204 0.94 (0.61-1.44)	
					Q5: 205+ n=2162, 36 diabetes (1.7%)/2126 non- diabetics	0.93 (0.59-1.45)
				urine As (µg/L, model adjusted for creatinine)	Q1: 1-36 µg/L urine n=2184, 44 diabetes (2.0%)/2140 non- diabetics	OR=1.00
					Q2: 37-66 1.44 (0.97-2.17)	
					Q3: 67-114 n=2184, 44 diabetes (2.0%)/2140 non- diabetics	1.20 (0.77-1.85)
					Q4: 115-204 1.16 (0.73-1.85)	
					Q5: 205+ n=2184, 44 diabetes (2.0%)/2140 non- diabetics	1.22 (0.73-2.03)
			glucosuria	time weighted	Q1: 0-8.0 µg/L water OR=1.00	

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For																												
			prevalence based on results from colormetric test strips, also no evidence of an association based on glycosylated hemoglobin (HbA1c) in a subset of 2,100 participants	water As (µg/L) urine As (µg/L) urine As (µg/L, model adjusted for creatinine)	<table border="1"> <tr><td>Q2: 8.1-41.0</td><td>1.07 (0.71-1.63)</td></tr> <tr><td>Q3: 41.2-91.7</td><td>1.12 (0.74-1.71)</td></tr> <tr><td>Q4: 91.8-176.1</td><td>1.01 (0.66-1.56)</td></tr> <tr><td>Q5: 176.2-864</td><td>1.20 (0.79-1.81)</td></tr> <tr><td>Q1: 1-36 µg/L urine</td><td>OR=1.00</td></tr> <tr><td>Q2: 37-66</td><td>0.91 (0.62-1.34)</td></tr> <tr><td>Q3: 67-114</td><td>0.79 (0.52-1.19)</td></tr> <tr><td>Q4: 115-204</td><td>0.65 (0.43-1.01)</td></tr> <tr><td>Q5: 205+</td><td>0.91 (0.60-1.37)</td></tr> <tr><td>Q1: 1-36 µg/L urine</td><td>OR=1.00</td></tr> <tr><td>Q2: 37-66</td><td>0.95 (0.63-1.42)</td></tr> <tr><td>Q3: 67-114</td><td>0.85 (0.55-1.31)</td></tr> <tr><td>Q4: 115-204</td><td>0.72 (0.45-1.15)</td></tr> <tr><td>Q5: 205+</td><td>1.03 (0.63-1.68)</td></tr> </table>	Q2: 8.1-41.0	1.07 (0.71-1.63)	Q3: 41.2-91.7	1.12 (0.74-1.71)	Q4: 91.8-176.1	1.01 (0.66-1.56)	Q5: 176.2-864	1.20 (0.79-1.81)	Q1: 1-36 µg/L urine	OR=1.00	Q2: 37-66	0.91 (0.62-1.34)	Q3: 67-114	0.79 (0.52-1.19)	Q4: 115-204	0.65 (0.43-1.01)	Q5: 205+	0.91 (0.60-1.37)	Q1: 1-36 µg/L urine	OR=1.00	Q2: 37-66	0.95 (0.63-1.42)	Q3: 67-114	0.85 (0.55-1.31)	Q4: 115-204	0.72 (0.45-1.15)	Q5: 205+	1.03 (0.63-1.68)	
Q2: 8.1-41.0	1.07 (0.71-1.63)																																	
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Q4: 91.8-176.1	1.01 (0.66-1.56)																																	
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Q5: 205+	1.03 (0.63-1.68)																																	
case-control Mexico (Coronado-Gonzalez <i>et al.</i> 2007) environmental, low to moderate	400 subjects from an endemic region of Coahuila, Mexico, a region in Northern Mexico with high incidence of diabetes (31.5% male in cases and 38% in controls; ≥30 years of age)	200 cases/200 non-cases	diabetes (2 fasting glucose values of ≥126 mg/100 ml, history of diabetes treated with insulin or oral hypoglycemic drugs)	urinary As (ug/g creatinine) *water iAs of 20-400µg/L	<table border="1"> <tr><td><63.5 µg/g creatinine</td><td>OR=1.00</td></tr> <tr><td>63.5-104</td><td>2.16 (1.23-3.79)</td></tr> <tr><td>≥104</td><td>2.84 (1.64-4.92)</td></tr> </table>	<63.5 µg/g creatinine	OR=1.00	63.5-104	2.16 (1.23-3.79)	≥104	2.84 (1.64-4.92)	age, sex, hypertension, family history, obesity, and serum lipids																						
<63.5 µg/g creatinine	OR=1.00																																	
63.5-104	2.16 (1.23-3.79)																																	
≥104	2.84 (1.64-4.92)																																	
cross-sectional Mexico (Del Razo <i>et al.</i> submitted) environmental, low to moderate	258 subjects from the arsenic endemic regions of Zimapan (n=147) and Lagunera (n=111) (33% male; 5-88 years of age, average of 34 years)	FBG ≥126 mg/dL: 23 cases/235 non-cases 2hour BG ≥200: 9 cases/249 non-cases diabetes medication or doctor diagnosis: 17 cases/241 non-	diabetes (FBG ≥126 mg/dL, 2-hour BG ≥200 mg/dL, HbA1c > 7%, existing diagnosis, use of anti-diabetic medication)	drinking water As (mean = 42.9 µg/L; range of 3.1-215.2 µg/L)	<table border="1"> <tr><td>FBG≥126 mg/dL: per 10 µg/L increase in iAs</td><td>OR=1.15 (1.06–1.24)</td></tr> <tr><td>FBG≥126: >125 vs 10 µg/L</td><td>5.01 (1.02-24.7)</td></tr> <tr><td>2HBG≥200mg/dL: per 10 µgl/L increase in iAs</td><td>OR=1.14 (1.05–1.24)</td></tr> <tr><td>HbA1c</td><td>β=0.019 (0.002–0.037)</td></tr> <tr><td>FPI</td><td>β= -0.208 (-0.272– -0.145)</td></tr> <tr><td>HOMA-IR</td><td>β= -0.164 (-0.236– -0.092)</td></tr> </table>	FBG≥126 mg/dL: per 10 µg/L increase in iAs	OR=1.15 (1.06–1.24)	FBG≥126: >125 vs 10 µg/L	5.01 (1.02-24.7)	2HBG≥200mg/dL: per 10 µgl/L increase in iAs	OR=1.14 (1.05–1.24)	HbA1c	β=0.019 (0.002–0.037)	FPI	β= -0.208 (-0.272– -0.145)	HOMA-IR	β= -0.164 (-0.236– -0.092)	age, sex, obesity, and hypertension																
FBG≥126 mg/dL: per 10 µg/L increase in iAs	OR=1.15 (1.06–1.24)																																	
FBG≥126: >125 vs 10 µg/L	5.01 (1.02-24.7)																																	
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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For
		cases		urine total As (mean = 41.2 ng/mL; range of 2.3-233.7 ng/mL)	FBG≥126 mg/dL: per 10 ng/mL increase in iAs OR=1.004 (0.995–1.014)	
	HbA1c > 7%: 26 cases/232 non-cases		insulin sensitivity [fasting plasma insulin (FPI), HOMA-IR]		2HBG≥200mg/dL: per 10 ng/mL increase in iAs OR=1.003 (0.994–1.013)	
					HbA1c β=0.004 (-0.015–0.023)	
					FPI β= -0.136 (-0.207– -0.066)	
					HOMA-IR β= -0.116 (-0.193– -0.040)	
				urine DMA ^{III}	FBG≥126 mg/dL: per 10 ng/mL increase in iAs OR=1.047 (1.000–1.097)	
					2HBG≥200mg/dL: per 10 ng/mL increase in iAs OR=1.051 (1.002–1.101)	
					HbA1c β=0.005 (-0.007–0.016)	
					FPI β= -0.085 (-0.128– -0.042)	
					HOMA-IR β= -0.063 (-0.110– -0.017)	
retrospective cohort US (Enterline and Marsh 1982) occupational	Cause of death through 1976 in 2802 men who worked for ≥1 year at a Tacoma, WA copper smelter between 1940–1964 (1,061 total deaths; <20-64 years of age at hire)	1061 deaths 12 diabetes deaths (1.1%)/ 1049 other	diabetes (death certificate)	job title	smelter workers vs. white men for US population SMR=0.85 (0.46-1.44)¹	age
prospective cohort US (Ettinger <i>et al.</i> 2009) environmental, low to moderate	532 pregnant women living proximate to the Tar Creek, OK superfund site (14-36+ years of age)	~64 cases (12%/ 468 non-cases	impaired glucose tolerance during pregnancy (28 weeks OGTT, elevated glucose >140 mg/dL)	blood As (µg/L), n=456 and 439 when subjects with history of diabetes excluded	Q1 (0.23-0.92) Q2 (0.93-1.39) Q3 (1.40-2.08)	age, pre-pregnancy BMI, ethnicity/race, Medicaid use, married or living with partner
					all subjects: OR=1.00 no history diabetes: OR=1.00	
					all subjects: 1.02 (0.39-2.69) no history diabetes: 1.07 (0.39-2.98)	
					all subjects: 2.65 (1.12-6.36) no history diabetes: 2.83 (1.14-7.02)	

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For
					Q4 (2.09-24.07)	all subjects: 2.79 (1.13-6.87) no history diabetes: 2.46 (0.91-6.62)
					<i>p</i> -trend	all subjects: 0.008 no history diabetes: 0.04
				hair As (ng/g), n=149 and 143	Q1 (1.10-8.81)	all subjects: 1.00, OR no history diabetes: 1.00, OR
				when subjects with history of diabetes excluded	Q2 (8.93-13.11)	all subjects: 3.97 (0.62-25.37) no history diabetes: 4.51 (0.36-56.55)
					Q3 (13.26-24.12)	all subjects: 5.77(0.98-33.88) no history diabetes: 13.24 (1.27-138)
					Q4 (24.22-724.41)	all subjects: 4.20 (0.74-23.86) no history diabetes: 8.62 (0.87-85.20)
					<i>p</i> -trend	all subjects: 0.4 no history diabetes: 0.11
cross-sectional Denmark (Jensen and Hansen 1998) occupational	32 male workers occupationally exposed to arsenic (e.g., taxidermists, wood workers, other jobs), 6 male colleagues of workers, and 26 unexposed (64 total subjects; reference group, 85% male; mean age was 37 years, range of 20-60 years)	64 subjects 5 diabetics (7.8%)/ 59 non-diabetics	diabetes (HbA1c levels >7%)	job title	As exposed workers vs. colleagues + a reference group with no known arsenic exposure	RR=4.43 (0.47-42.0)² *median HbA1c was significantly higher in As workers compared to reference group (5.4 versus 4.4 %of total hemoglobin)

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
cross-sectional Sweden (Lagerkvist and Zetterlund 1994) occupational	Medical symptoms in 43 male smelter workers and 46 male referents during the period of 1982-1987 (89 total subjects; mean age: 59 in smelter workers; 57 in referents)	89 subjects exp: 4 diabetes (9.3%)/39 other ref: 0 diabetes (0%)/46 other	diabetes (self-reported)	job title	smelter workers vs. workers from a nearby car factory with no known arsenic exposure	RR=9.61 (0.53-173)²	crude
cross-sectional Taiwan (Lai <i>et al.</i> 1994) environmental, HAA	Diabetes in 891 residents in arseniasis-endemic area of southern Taiwan (43% male; 30-60+ years of age)	86 cases/805 non-cases	diabetes (OGTT, self-reported history of treatment for diabetes)	CEI drinking water	0 (n=108) 1 (0.9%)/107 0.1–15 ppm-yrs ≥15 ppm-yrs (n=326) 47 (14.4%)/279 unknown	OR=1.00 6.6 (0.9–51.0) 10.1 (1.30–77.9) 5.7 (0.71–45.5)	age, sex, BMI, physical activity
retrospective cohort USA (Lewis <i>et al.</i> 1999) environmental, low to moderate	Cause of death in 2,203 deceased members of a larger cohort (n=4,058) of Millard County, UT residents (52% male; 50-80+ years of age) total deaths: 2,203 men: 1242 deaths women: 961 deaths	55 diabetes deaths/ 2148 other deaths men: 20/1222 women: 35/926	diabetes (death certificate)	CEI community drinking water: (number of years of residence in community + survey data on median As levels in public and private drinking water samples). *median drinking water As for towns ranged from 14-166 ppb	SMRs, residents of Millard County, Utah vs white male and female from general population of Utah (1960-1992) low (<1 ppm-yr) medium (1– < 5 ppm-yr) high (≥5 ppm-yr) all	male: 0.93 female: 1.14 male: 0.95 female: 1.72 male: 0.42 female: 0.89 male: 0.79 (0.48-1.22) female: 1.23 (0.86-1.71)	sex, race

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For
retrospective cohort USA (Lubin <i>et al.</i> 2000) occupational	Cause of death between 1938–1959 in 8014 men who worked for ≥1 year prior to 1957 at a MT copper smelter (5011 total deaths; <20-30+ years of age at hire)	5,011 deaths 54 diabetes deaths (1.1%)/ 4957 other	diabetes (death certificate)	job title	smelter workers vs. US mortality rates SMR=0.83 (0.63-1.08)	age
retrospective cohort USA (Mabuchi <i>et al.</i> 1980) occupational	Cause of death in 1,393 pesticide workers in Baltimore, MD during the period of 1946–1977 (240 total deaths; 75% male; <20-40+ years of age at hire)	240 deaths 2 diabetes deaths (0.8%)/ 238 other	diabetes (death certificate)	job title	pesticide workers vs. US general population for “whites” pesticide workers vs. Baltimore city SMR=0.63 (0.10-2.07) ² men: 0.46 female: 1.03 SMR=0.47 (0.12-1.88)² men: 0.31 female: 0.63	age, sex, period
cross-sectional USA (Meliker <i>et al.</i> 2007) environmental, low to moderate	Cause of death in people residing in 6 contiguous counties of southeastern Michigan compared to statewide mortality rates (1979-1997; ≥35 years of age)	2861 diabetes deaths/ 77,143 non-diabetes deaths 41,282 total deaths, men 1,249 (3.0%)/40,033 38,722 total deaths, women 1,612 (4.2%)/37,110	diabetes (death certificate)	population-weighted As levels from 9,251 well water samples tested from 1983-2002 in the 6 counties and another 23,691 samples from the rest of the state <u>6 counties:</u> 11µg/L (mean) 7.6µg/L median) <u>rest of the state:</u> 2.98µg/L (mean) 1.3µg/L median)	residents of 6 counties vs. statewide mortality rates SMR men: 1.28 (99% CI 1.18-1.37) women: 1.27 (99% CI 1.19-1.35)	age, race

Appendix Tables
(version updated January 3, 2011)

Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
cross-sectional USA (Navas-Acien <i>et al.</i> 2008) environmental, low to moderate	NHANES 2003-2004, 788 adults (49.4% male, ≥20 years of age)	93 cases/695 non-cases	fasting serum glucose level ≥126 mg/dL, or self-reported physician diagnosis or use of insulin or oral hypoglycemic medication	total urinary As ($\mu\text{g/L}$)	<math><4.8 \mu\text{g/L}</math> (29 cases/202 non-cases)	OR=1.00	sex, age, race, and urine creatinine, education, BMI, serum cotinine level, hypertension medication, urine arsenobetaine, blood mercury levels
				*OR analyses also conducted based on urinary DMA and arsenobetaine (no statistically significant associations reported)	4.8-10.8 (30 cases/230 non-cases)	1.27 (0.36-4.48)	
					>10.8 (34 cases/263 non-cases)	1.60 (0.46-5.54) *p for trend = 0.03	
					80 th vs 20 th percentile (18.3 vs. 3.5 $\mu\text{g/L}$)	3.58 (1.18-10.83)	
					ratio of total arsenic in diabetics (6.2 $\mu\text{g/L}$) and non-diabetics (7.3 $\mu\text{g/L}$)	1.26 (1.02-1.56)	
cross-sectional USA (Navas-Acien <i>et al.</i> 2009a) environmental, low to moderate	NHANES 2003-2006, 1279 adults (% male not reported, ≥20 years of age)	160 cases/ 1119 non-cases	fasting serum glucose level ≥126 mg/dL, or self-reported physician diagnosis or use of insulin or oral hypoglycemic medication	total urinary As ($\mu\text{g/L}$)	80th vs 20th percentile (3.4 vs. 17.2 $\mu\text{g/L}$)	OR=2.86 (1.23-6.63)	sex, age, race, and urine creatinine, education, BMI, serum cotinine level, hypertension medication, blood mercury levels
				* multiple regression model adjusted for urine arsenobetaine	≥80th vs ≤20th	1.78 (0.60–5.30)	
					ratio in diabetics and non-diabetics	1.21 (1.06–1.38)	
			159/1109	total urine As minus arsenobetaine and arsenocholine ($\mu\text{g/L}$)	80th vs 20th percentile (2.5 vs. 11 $\mu\text{g/L}$)	1.72 (0.85–3.45)	*urine arsenobetaine/seafood consumption adjusted for by multiple approaches
				≥80th vs ≤20th	1.04 (0.30–3.59)		
				ratio in diabetics and non-diabetics	1.22 (1.00–1.48)		
	62/319	total As ($\mu\text{g/L}$ in participants with	80th vs 20th percentile (7.4 vs. 1.6 $\mu\text{g/L}$)	2.60 (1.12–6.03)			
			≥80th vs ≤20th	4.26 (0.83–21.8)			

Appendix Tables
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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
				undetetectable arsenobetaine concentrations, <0.4µg/L	ratio in diabetics and non-diabetics 1.28 (1.07–1.53)		
cross-sectional Bangladesh (Nabi <i>et al.</i> 2005) environmental, HAA	Diabetes in 115 patients diagnosed with arsenicosis and 120 control residents in the northwestern district of Chapainowabganj (235 total subjects; 49.8% male; 14-85 years of age)	~16 cases/219 controls	blood glucose >140 mg/dL	arsenicosis patient based on skin lesions	patient vs. control average As drinking water levels: 218.1 vs. 11.3 µg/L average As urine levels: 234.6 vs. 29.4 µg/L	RR=3.13 (1.04 to 9.42) ¹ prevalence ratio = 2.8 (10% of arsenicosis patients had diabetes versus 3.6% of controls)	crude
case-control Sweden (Rahman and Axelson 1995) occupational	Diabetes-related deaths in 369 men (116 who had been employed in copper smelter and 253 who had not been employed at smelter). Cases were people with diabetes listed on death record and supported with clinical information. Controls were people without diabetes listed. Subjects were 30-74 years of age at death, 43 total deaths. Subjects were also excluded from control group if death record listed arsenic-associated disease (cancer, cardio- or cerebrovascular disease)	43 deaths exp: 10 diabetes (37%)/17 other* ref: 2 diabetes (12.5%)/14 other* *“other” = deaths from other than diabetes, cancer, cardio- or cerebrovascular disease	diabetes (death certificate supported with clinical information)	air levels	0 mg/m ³ << 0.5 mg/m ³ ~0.5 mg/m ³ >0.5 mg/m ³ total exposed	OR = 1.00 2.0 (0.1-27) 4.2 (0.3-54) 7.0 (0.7-79) Mantel-Haenszel OR (95% CI) 3.30 (0.50-30.0)	crude age
						*unistratified test for trend, X ² (1) =4.68, p=0.03	

Appendix Tables
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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For
case-control Sweden (Rahman <i>et al.</i> 1996) occupational	Diabetes-related deaths in 5498 men based on death and burial registry during 1950-1982 (888 of the men were glass workers with likely As exposure and 4610 men were considered unexposed as a reference group). Cases were people with diabetes listed on death record and supported with clinical information. Controls were people without diabetes listed. Subjects were 45-75+ years of age; 2456 total deaths. Subjects were also excluded from control group if death record listed an arsenic-associated disease (cancer, cardio- or cerebrovascular disease)	2,333 deaths exp: 31 diabetes (12.9%)/209 other* ref: 199 diabetes (10.5%)/1894 other* *“other” = deaths from other than diabetes, cancer, cardio- or cerebrovascular disease	diabetes (death certificate)	job title	glass workers vs. others included in the registry who were not glass workers OR=1.40 (0.90-2.10)	age
cross-sectional Bangladesh (Rahman <i>et al.</i> 1998) environmental, HAA	Diabetes in 163 arsenic exposed and 854 unexposed people (1017 total subjects; 59% male; 30-60+ years of age)	total cohort: 46 cases/971 non-cases	self-reported symptoms, previous diagnosis, glucosuria, OGTT	living in HAA and keratosis time weighted water concentration ⁵	unexposed (non HAA, no keratosis) exposed (HAA, keratosis) unexposed “low” (<0.5 mg/L) “medium” (0.5–1 mg/L) “high” (>1 mg/L) PR=1.00 5.2 (2.5–10.5) PR=1.00 2.6 (1.2–5.7) 3.9 (1.8–8.2) 8.8 (2.7–28.4)* *p for trend < 0.001	age, sex, BMI; 3 keratotic and diabetic cases from one family excluded from some analyses to avoid biasing risk results same as above except BMI was not adjusted for (appeared to be negative confounder)

Appendix Tables
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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
cross-sectional Bangladesh (Rahman <i>et al.</i> 1999) environmental, HAA	Diabetes in 1481 arsenic exposed and 114 unexposed residents (1595 total subjects; 61% male; 30-60+ years of age)	263 cases/1332 non- cases in total cohort	glucosuria in subjects with and without skin lesions	time	unexposed	PR=1.00	age and sex
				weighted	"I" (<0.5 mg/L)	no skin lesion 0.8 (0.4– 2.3)	
				concentratio n ⁵	"II" (0.5–1 mg/L)	skin lesion 1.1 (0.5–2.0) no skin lesion 1.4 (0.8– 2.3)	
					"III" (>1 mg/L)	skin lesion 2.2 (1.3–3.8) no skin lesion 1.4 (0.7– 2.4)	
						skin lesion 2.6 (1.5–4.6) *p for trend < 0.01	
					unexposed	PR=1.00	
				CEI village drinking water	<1.0 mg-years/L	no skin lesion 0.4 (0.1– 1.0)	
					1–5 mg-years/L	skin lesion 0.8 (0.3–1.9) no skin lesion 0.9 (0.5– 1.7)	
					>5–10 mg-years/L	skin lesion 1.7 (0.9–2.9) no skin lesion 1.2 (0.6– 2.2)	
					>10 mg-years/L	skin lesion 2.1(1.0–4.0) no skin lesion 1.7 (1.0– 2.9)	
	10 vs 0 ppm-year	skin lesion 2.9 (1.6–5.2) *p for trend < 0.001 2.10 (1.10–4.20) ²					
case-control Spain (Ruiz-Navarro <i>et al.</i> 1998) environmental, low to moderate	38 hospital patients with diabetes (gender not reported) and 49 healthy individuals (87 total subjects; 39% male; age not reported)	38 cases/49 non-cases	NR	urine As (µg/L)	75 th vs. 25 th percentile	0.87 (0.50-1.53)² *no difference in mean urinary As concentrations between diabetics (3.44 µg/L) and healthy subjects (3.68 µg/L)	crude

Appendix Tables
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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For		
cross-sectional USA (Steinmaus <i>et al.</i> 2009a) environmental, low to moderate	NHANES 2003-2004, 795 adults (53% male, ≥20 years of age)	98 cases/697 non- cases	<u>diabetes</u> fasting serum glucose level ≥126 mg/dL, or self-reported physician diagnosis or use of insulin or oral hypoglycemic medication	“estimated inorganic” As (µg/L), based on subtracting organic arsenic (arsenobetaine and arsenocholine) from the concentration of total arsenic	≤4.1 µg/L (36 cases/227 non- cases) 4.2-8.5 (29 cases/238 non- cases) >8.5 (33 cases/232 non- cases) ≥80th vs ≤20th (n=323) 42 cases (13%)/281 non-cases) 11.9 vs. 2.7 µg/L	OR=1.00 0.63 (0.34–1.15) 0.98 (0.53–1.80) 1.15 (0.53–2.50)	sex, age, ethnicity, education, BMI, serum cotinine, urine creatinine, current use of hypertension medications	
				total urinary As (µg/L) and including adjustment for arsenobetaine to replicate Navas-Acien (2008)	80th vs 20th	3.57 (1.28–9.95)		sex, age, ethnicity, education, BMI, serum cotinine, current use of hypertension medications, arsenobetaine
				total urinary As (µg/L)	≤5.2 µg/L (36 cases/232 non- cases)	OR=1.00		sex, age, ethnicity, education, BMI, serum cotinine, current use of hypertension medications
				*OR analyses also conducted based on	5.3-11.8 (32 cases/230 non- cases)	0.87 (0.48–1.55)		sex, age, ethnicity, education, BMI, serum cotinine, current use of hypertension medications
				urinary DMA and arsenobetaine	>11.8 (30 cases/235 non- cases)	0.76 (0.42–1.39)		sex, age, ethnicity, education, BMI, serum cotinine, current use of hypertension medications
				(no statistically significant associations reported)	≥80th vs ≤20th (18.3 vs. 3.5 µg/L; 38 cases/283 non-cases)	0.88 (0.39–1.97)		

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
cross-sectional USA (Steinmaus <i>et al.</i> 2009b) environmental, low to moderate	NHANES 2003-2006 (≥20 years of age)	NR	fasting serum glucose level ≥126 mg/dL, or self-reported physician diagnosis or use of insulin or oral hypoglycemic medication	total As in participants with undetectable arsenobetaine concentrations	≥80th vs ≤20th	OR= 1.03 (0.38–2.80)	sex, age, race, BMI
						2.32 (0.51–10.5)	sex, age, race, BMI, urine creatinine as continuous variable
						1.19 (0.10–14.0)	sex, age, race, BMI, urine creatinine as categorical variable
retrospective cohort USA (Tollestrup <i>et al.</i> 2003) occupational	Cause of death in 3,132 subjects (1,827 boys and 1,305 girls) who lived within 4 km (2.5 miles) of a Ruston, WA copper smelter and arsenic refinery for at least 2 years between 1907-1932 (1074 total deaths; 58% male; <14 years of age at exposure); follow-up status determined in 1990	16 diabetes deaths/1058 non-diabetes deaths	diabetes (death certificate)	number of years living < 1.6 km (1.0 miles) of from smelter stack	<1 versus ≥ 10 years	RR=1.60 (0.36-7.16)²	crude
					0 – <1 years	men: 1.4 (0.3-4.0) female: 0.0	crude death rate per 10,000 person years
					1–3.9 years	men: 2.0 (0.4-5.8) female: 4.3 (1.2-10.9)	
					4.0–9.9 years	men: 0.0 female: 2.0 (0.2-7.1)	
					≥ 10 years	men: 2.4 (0.5-7.0) female: 0.8 (0.0-4.3)	
retrospective cohort Taiwan (Tsai <i>et al.</i> 1999) environmental, HAA	Diabetes-related deaths from 20,067 death records in a blackfoot endemic areas (1971-1994) compared to local and national reference groups (all ages)	531 diabetes deaths/19,536 non-diabetes deaths	diabetes (death certificate)	living in HAA	blackfoot endemic area vs. local reference	SMR=1.47 (1.35-1.60)¹ men: 1.35 (1.16-1.55) female: 1.55 (1.39-1.72)	age, sex
					blackfoot endemic area vs. national reference	SMR=1.20 (1.10-1.30)¹ men: 1.14 (0.98-1.31) female: 1.23 (1.11-1.37)	
prospective	Development of diabetes in 446	41 cases/405 non-	fasting glucose,	CEI drinking	<17 mg/L-yr	RR=1.00	age, sex, BMI

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
cohort Taiwan (Tseng <i>et al.</i> 2000a; Tseng <i>et al.</i> 2000b) environmental, HAA	non-diabetic residents in southwestern arseniasis-endemic area (50% male; >30 years of age, mean of 47.5)	cases	OGTT	water	≥ 17 mg/L-yrs	2.1 (1.1-4.2)	
cross-sectional Taiwan (Wang <i>et al.</i> 2003) environmental, HAA	Diabetes in 66,667 residents in southwestern arseniasis-endemic area and 639,647 ³ residents in non-endemic area (706,314 total subjects; 43% male; 25-65+ years of age)	27,543 cases/ 678,771 non-cases	reimbursement claims from the National Health Insurance Database for clinical diagnosis and medical prescriptions	living in HAA	<u>non-endemic area</u> <u>endemic area</u>	OR =1.00 2.69 (2.65–2.73)	age, sex
cross-sectional Taiwan (Wang <i>et al.</i> 2007) environmental, low to moderate	660 residents of central Taiwan 2002-2003 (complete information available for 432 residents; 44% male; 35-64 years of age)	214 cases/446 non-cases based on 660 residents; diabetes cases among the final 432 participants was not reported	metabolic syndrome defined as ≥3 out of 5 parameters: 1. triglycerides ≥150mg/dl 2. HDL ♀: <50, ♂: <40 mg/dl 3. systolic BP ≥130 mmHg or diastolic BP ≥ 85 4. BMI ♀F: ≥25, ♂ ≥ 27 5. glucose ≥110 mg/dl	hair As (µg/g) *0.06 µg/g in industrial areas vs. 0.04 µg/g in non-industrial areas, p=0.002)	<u>“low”</u> <u>“median”</u> <u>“high”</u>	OR=1.00 <u>2.54 (1.2-5.39)</u> 2.35 (1.02-5.43) *authors also reported significant linear relationship between hair As and plasma glucose, lipids, and blood pressure	age, sex, occupation, lifestyle factors (alcohol, betel nut chewing, smoking, groundwater use)

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For	
cross-sectional China (Wang <i>et al.</i> 2009) environmental, low to moderate	111 villagers living in arsenic-endemic areas and 124 living in a control area (40% and 48% male, respectively; >30 years of age)	endemic areas: 57 cases/54 non-cases control areas: 72 cases/52 non-cases	local hospital records followed by clinical examinations	urine (µg/g creatinine)	control area: diabetics (192 µg/g) vs. non-diabetics (231 µg/g) <hr/> endemic area: diabetics (328 µg/g) vs. non-diabetics (209 µg/g)	no significant difference in means RR = 1.03 (0.97-1.09) ¹ <hr/> no significant difference in means RR = 1.10 (0.98-1.23)¹	crude
case-control UK (Ward and Pim 1984) environmental, low to moderate	87 diabetic patients from Oxford, England (32 insulin-dependent, 55 non-insulin dependent) and 30 non-diabetic individuals (117 total subjects; 65% male; 18-78 years of age)	87 cases/30 non-cases	NR	plasma As (µg/mL)	75 th vs. 25 th percentile	1.09 (0.79-1.49)² * mean plasma As concentrations were significantly higher in insulin dependent diabetics (0.018 µg/mL) and non-insulin dependent diabetics (0.200 µg/mL) compared to healthy subjects (0.015 µg/mL)	crude
cross-sectional USA (Zierold <i>et al.</i> 2004) environmental, low to moderate	1185 residents (average of 62 years of age) from 19 townships in areas of WI with elevated groundwater As (nearly 20% of samples > 10 µg/L)	67 cases/1118 non-cases ⁴	self-report	drinking water As (ppb or µg/L), private well-water that subjects reported drinking for 20-83 years	<2 µg/L <hr/> 2 to ≥10 <hr/> >10	OR=1.00 1.35 (0.78-2.33) 1.02 (0.49-2.15)	age, sex, BMI, smoking

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Appendix Table A. Epidemiology studies of arsenic and diabetes, glucose tolerance, or metabolic syndrome

Study Design	Population	N	Diagnosis	Exposure Assessment	Findings	Adjusted For
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¹Risk estimates calculated based on data presented in the paper using an open source epidemiology statistics programs, OpenEpi (<http://www.openepi.com/menu/openEpiMenu.htm>) or MedCalc (http://www.medcalc.be/calc/relative_risk.php)

²Relative risk and 95% confidence interval as estimated by Navas-Acien et al. (2006)

³ There appears to be an error in Wang *et al*, (2003) on the number of people included in the “non-endemic” area category based on the n’s provided in Table 1

⁴ Number of cases were not reported in original study by Zierold et al. (2004) but were reported in Navas-Acien et al (2006)

⁵Although the arsenic water concentrations in Rahman et al. (1998) are expressed units of mg/L, the value is supposed to represent the “Approximate time-weighted mean arsenic exposure levels were calculated over the lifetime of each subject as $2\gamma(a, -C_{jy}Z_{jdj}$, where a_j is the number of years a well with arsenic concentration c_{jy} was used, assuming that the current levels of arsenic in the well water were also representative of the past.”

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Appendix Tables
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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>(Aguilar <i>et al.</i> 1997) Wistar rats (male weanlings); 0 or 5 µg/g dietary arsenic pentoxide, As(V) oxide or As₂O₅ [~2.5 mg/kg bw/d based on 0.05 kg diet consumption/kg bw/d by male weanling Wistar rats] <u>Also presented in study, but not summarized here:</u> Tissue arsenic levels and organ weights for both As(V) and co-treatment group of As(V) + chromium III</p>	5	[~0.25] As(V)	<p>↑ plasma cholesterol (~2-fold); effects on cholesterol not observed following co-treatment with 5 µg/g dietary chromium III (as CrCl₃·6H₂O) <u>Not affected:</u> body weight, feed and water intake, plasma glucose</p>
<p>(Arnold <i>et al.</i> 2003) Fisher F344 rats; 0, 50, 400, or 1300/1000/800* ppm dietary monomethylarsonic acid (MMA) for 2-years. Authors calculated ppm to mg/kg bw/day doses: 50 ppm = males 3–6.9, females 3.9–7; 400 ppm = males 25.7–57, females 33.9–54.5; 1300 ppm = males 96.7–178.6, females 116.8–174.4; 1000 ppm = males 70.4, females 85.3; 800 ppm = males 65.8, females 67.6] * top dose reduced during study</p> <p><u>Also presented in study, but not summarized here:</u> Other chronic toxicity/carcinogenicity outcomes not related to blood glucose levels (urinary glucose not assessed/reported) or pancreatic effects</p>	60/sex	<p>[3–178.6] MMA (males)</p> <hr/> <p>[3.9–174.4] MMA (females)</p>	<p>pancreatitis (significant trend, P < 0.001); ↓ blood glucose in high dose group at 12 months only</p> <hr/> <p>pancreatitis (significant trend, P < 0.001); ↓ blood glucose in high dose group at 12 months only <u>Not affected at any dose level in either sex:</u> incidence of pancreatic islet cell adenomas or carcinomas in any treatment group</p>
<p>(Arnold <i>et al.</i> 2003) B6C3F1 mice; 0, 10, 50, 200 or 400 ppm diet monomethylarsonic acid (MMA) for 2-years. Authors calculated ppm to mg/kg bw/day doses: 10 ppm = males 1.2–3.1, females 1.4–4; 50 ppm = males 6.0–15.5, females 7.0–19.5; 200 ppm = males 24.9–62.1, females 31.2–75.7; 400 ppm = males 67.1–128, females 101–155.7]</p> <p><u>Also presented in study, but not summarized here:</u> Other chronic toxicity/carcinogenicity outcomes not related to the pancreas (blood or urinary glucose not assessed/reported)</p>	52/sex	<p>[1.2-128] MMA (males)</p> <p>[1.4-155.7] MMA (females)</p>	<p><u>Not affected at any dose level in either sex:</u> incidence of pancreatic islet cell adenomas or carcinomas in high dose groups (blood glucose not assessed)</p>

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>(Biswas <i>et al.</i> 2000) Black Bengal goats (female); 0 or 25 mg/kg bw/d sodium arsenite, As(III), orally by capsule daily for 12 weeks (administered dose is one-fifth of acute LD₅₀ in goats) <u>Also presented in study, but not summarized here:</u> tissue histopathology (effects on pancreas not assessed/reported); serum protein; liver enzymes; mortality and tissue arsenic levels</p>	6	25 As(III)	↑ blood glucose beginning week 6 (1.3-1.4-fold); ↓ body weight gain during weeks 9 and 12
<p>(Boquist <i>et al.</i> 1988) C57BL+/+ mice (adult male and female); 0 or 10 mg/kg bw arsenite, As(III), by ip injection (single dose) <u>Also presented in study, but not summarized here:</u> Study was designed to assess effects of compounds that can inhibit citric acid cycle enzyme activity (arsenite, asparagine, fluoroacetate, hydroxylamine, malonate, and methionine) on pancreatic islet cell histology, blood glucose, and insulin release. Only arsenite-related effects summarized here.</p>	5-10	10 As(III)	↓ blood glucose from 4 hours post dose through 1 day after injection (20 – 37% of control, “delayed hypoglycemia”); hepatic glycogen depletion and microvesicular fatty change; ↓ glucose-induced (18 mM) insulin release (35% of control).
<p>(Cobo and Castineira 1997) Wistar rats (adult male); 0 or 17.75-100* mg/L arsenite, As(III) as As₂O₃ in the drinking water; *Concentrations increased from 17.75 mg/L during the first week to 100 mg/L during the 8th week [~1.68-9.5 mg/kg bw/d based on fluid consumption of 0.095 L/kg bw/d by male adult Wistar rats] <u>Also presented in study, but not summarized here:</u> Effects of chromium supplementation (Chromium (III) as CrCl₃)</p>	7	[~1.68 – 9.5] As(III)	delayed drop in glucose during glucose tolerance test; ↑ glucose-induced insulin release from isolated islet cells (~1.13- fold at 2.8 mM glucose and ~1.2-fold at 16.7 mM glucose); authors note some inhibition of mitochondrial respiration (states 3 and 4) and effect on pancreatic amylase <u>Not affected at any dose level:</u> fasting insulin levels
<p>(Ghafghazi <i>et al.</i> 1980) CD rats (male); 0, 5, or 10 mg/kg bw/d sodium arsenite, As(III), by ip injection either as a single dose treatment (and assessed 1.5 or 3 hours after injection) or treated daily for 7 days (and assessed 24-hours after last treatment). Acute effects of 10 mg/kg bw As(III) on resting glucose also assessed in adrenalectomized rats</p>	4	5 As(III)	↑ blood glucose in glucose tolerance test (~1.1–1.6 –fold; “impaired glucose tolerance”) following 7-day treatment; ↑ resting blood glucose following single (~1.5-fold) but not 7-day treatment; altered mitochondrial respiration following 7-days of treatment based on ↓ pyruvate/malate mediated state 3 respiration; ↑ state 4 respiration following utilization of added ADP (no effect on mitochondrial respiration measured 1.5 or 3 hours following single treatment)
	4	10 As(III)	↑ blood glucose in glucose tolerance test (~1.1–1.6 –fold; “impaired glucose tolerance”); ↑ resting blood glucose following single treatment (~2-fold) and 7-days of treatment (1.09-fold); altered mitochondrial

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
			respiration following 7-days of treatment based on ↓pyruvate/malate mediated state 3 respiration; ↑ state 4 respiration following utilization of added ADP (no effect on mitochondrial respiration measured 1.5 or 3 hours following single treatment)
	4	10 As(III), single treatment (adrenalectomized rats)	As(III) effects assessed in adrenalectomized rats were either not observed (for resting hyperglycemia) or attenuated (the impairment of glucose tolerance) leading authors to conclude that As(III) effects on glycemic control are partly mediated through the adrenal glands
(Hill et al. 2009) Pregnant LM/Bc/Fnn mice (female); 0 or 9.6 mg/kg bw sodium arsenate, As(V), by ip injection on GD 7.5 and GD 8.5, evaluated on GD 9.0	10	9.6 As(V)	<p>↑ plasma glucose at all time points in a 2-hour glucose tolerance test (1.2 to 1.6-fold); ↑ HOMA-IR index (1.6-fold); ↑ non-fasted plasma glucose (~1.2-fold) and fasted plasma glucose (1.4-fold); no effect on insulin levels at initiation or 30 minutes following start of glucose tolerance test (an apparently normal insulin response in the glucose tolerance test); 100% incidence of neural tube defects.</p> <p>This 100% incidence of arsenate-induced neural tube defects was significantly reduced in animals co-treated with several “rescue” agents: N-acetyl cysteine (NAC), an antioxidant (38.2%); the insulin pellet LinBit (46.4%); methionine, a methyl donor (67.9%); N-tert-butyl-α-phenylnitron (PBN), a compound that traps and stabilizes oxidative radicals (74.1%); and sodium selenate (SS), the active center of antioxidant selenoenzymes such as glutathione peroxidase (76.6%). Despite the efficient “rescue” by NAC (from 100% to 38.2% neural tube defects) this treatment did not significantly alter arsenate-induced elevations in fasting plasma glucose or insulin (the impacts of methionine, PBN, and SS on fasting plasma glucose and insulin were not evaluated).</p> <p><u>Not affected:</u> body weight</p>

Appendix Tables
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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>(Izquierdo-Vega <i>et al.</i> 2006) Wistar rat (adult male); 0 or 1.7 mg/kg sodium arsenite, As(III), by gavage twice a day for 90 days [equivalent to 3.4 mg As(III)/kg bw/d]</p>	10	[3.4] As(III)	<p>↑ fasting serum glucose (1.8-fold); ↑ fasting blood insulin (2.6-fold); ↓ glucose:insulin ratio (45% of control) ; ↑ HOMA-IR (3.9-fold); ↓ serum glucagon (~90% of control); ↓ labeling of insulin in pancreatic β-cells; altered glucagon staining pattern in islet cells; effects on indicators of oxidative stress and damage in pancreas: ↑ glutathione, GSH (1.7-fold); ↓ thioredoxin reductase activity (88% of control); ↑ lipid peroxidation expressed as thiobarbituric acid reactive substances, TBARS (~1.35-fold)</p> <p>Arsenic concentrations were measured in pancreas (inorganic As (iAs), MMA, DMA, TMAO, and sum As); the sum As concentrations were 43-fold higher in treated animals compared to controls (11.2 versus 0.23 μg/g tissue). The most predominate form was DMA (57% of sum As in controls and 83% of sum As in treated animals); iAs was 50% of sum As in controls and 1.4% of sum As in treated animals)</p> <p><u>Not affected:</u> water or food consumption; body weight</p>
<p>(Judd 1979) <i>Peromyscus leucopus</i> (white footed mice – either field-trapped or the F1 generation of field-trapped mice) [sex not stated] received 0 or 1000 ppm Monosodium methanearsonate (MSMA; an arsenical pesticide containing As(V)) in the drinking water for 30 or 60 days. [1000 ppm dose is 124.5 mg/kg bw/d based on fluid consumption reported by the authors.]</p> <p><u>Also presented in study, but not summarized here:</u> Results of the acute toxicity (LD50) study; hematocrit and hemoglobin concentrations.</p>	12	[124.5] MSMA	<p>↓ blood glucose concentration after 30 days of drinking MSMA.</p> <p><u>No effect at any dose level:</u> Body weight</p>
<p>(Mitchell <i>et al.</i> 2000) Sprague-Dawley rat, B6C3F1 mice, Golden-Syrian hamster, Hartley guinea pig; 0, 0.1, or 1 mg/kg sodium arsenite, As(III), by ip injection (single injection; adult males)</p> <p><u>Also presented in study, but not summarized here:</u> Blood arsenic following treatment with sodium arsenite or sodium arsenate;</p>	3 3	0.1 As(III) 1 As(III)	<p>no effects</p> <p>↑ blood glucose at 1 and 2 hours after injection in Hartley guinea pig (~1.1 to 1.3-fold)</p> <p><u>Not affected:</u> blood glucose in Sprague-Dawley rat, B6C3F1 mice, Golden-Syrian hamster</p>

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>other indicators of acute systemic toxicity, e.g., blood hematocrit, creatinine, urea nitrogen, etc. (these indicators not assessed reported/assessed following treatment with sodium arsenate).</p> <p>(Mukherjee <i>et al.</i> 2006) Albino rat (male); 0 or 3 mg/kg bw/d arsenic trioxide, As(III) oxide, via “oral” treatment for 30 days [specific route not specified].</p>	5	3 As(III) oxide	<p>↓ pancreatic islet cell counts (35 to 50% decrease compared to control); ↑ pancreatic production of NO (~1.9-fold), MDA (~1.8-fold), and OH⁻ (~2.8-fold); ↓ pancreatic SOD (~50% of control) activity, ↓ GSH content (~55% of control), ↓ CAT activity (~50% of control); ↑ serum TNF-α (~1.4-fold) and IL-6 (~1.2-fold). Many of the effects were reversed with treatment of As(III) oxide + folic acid (36 µg/kg bw/d) and As(III) oxide + folic acid (36 µg/kg bw/d) + vitamin B12 (0.63 µg/kg bw/d)</p>
<p>(Pal and Chatterjee 2004b) Wistar rat (male); 0 or 5.55 mg/kg bw/d sodium arsenite, As(III), by ip injection for 30 days. A “rescue” group was treated with As(III) + N-acetylcysteine (NAC; 163.2 mg/kg bw/d by “oral” route) for the last 7 days of As(III) treatment.</p> <p><u>Also presented in study, but not summarized here:</u> liver and kidney weight; liver pyruvic acid; liver glycogen; liver lactate dehydrogenase (LDH); liver and kidney free amino acid nitrogen, glucose-6-phosphatase activity and tissue protein</p>	6	5.55 As (III)	<p>↓ blood glucose (69% of control level)</p> <p>NAC treatment prevented the hypoglycemic effect as well as other arsenic-induced effects on liver glycogen, liver pyruvic acid, and free amino acid nitrogen in liver and kidney</p>
<p>(Pal and Chatterjee 2004a) Wistar rat (male); 0 or 5.55 mg/kg bw/d sodium arsenite, As(III), by ip injection for 21 days. A “rescue” group was treated with As(III) + methionine (0.8% in diet) for the last 5 days of As(III) treatment.</p> <p><u>Also presented in study, but not summarized here:</u> liver and kidney weight; liver pyruvic acid; liver glycogen; liver and kidney free amino acid nitrogen, glutamate-oxaloacetate (GOT) activity, and glutamate-pyruvate transaminase (GPT) activity</p>	6	5.55 As (III)	<p>↓ blood glucose (57% of control level)</p> <p>methionine treatment prevented the hypoglycemic effect as well as several other As(III)-induced effects on liver pyruvic acid and free amino acid nitrogen, GOT activity, and GPT activity in liver and kidney</p> <p><u>Not affected:</u> body weight</p>

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>(Pal and Chatterjee 2005) Wistar rat (male); 0 or 5.55 mg/kg bw/d sodium arsenite, As(III), by ip injection for 30 days. A “rescue” group was treated with arsenic + melatonin (10 mg/kg bw/d by ip injection) for the last 5 days of As(III) treatment. <u>Also presented in study, but not summarized here:</u> liver and kidney weight; liver pyruvic acid; liver glycogen; liver lactate dehydrogenase (LDH); liver and kidney free amino acid nitrogen, glutamate-oxaloacetate (GOT) activity, glutamate-pyruvate transaminase (GPT) activity, and glucose-6-phosphatase activity</p>	6	5.55 As (III)	<p>↓ blood glucose (55% of control level); ↑ urinary glucose (3.33-fold) melatonin treatment attenuated the hypoglycemic effect as well as several other arsenic-induced effects <u>Not affected:</u> body weight</p>
<p>(Paul et al. 2007b) C57BL/6 mice (male); 0, 25, or 50 ppm sodium arsenite, As(III), in the drinking water for 8 weeks (the 25 ppm group ingested an average of 94.7 µg of As/day, the 50 ppm group ingested an average 125.3 µg of As/day [~ 4.7 or 6.25 mg As(III)/kg bw/day]) <u>Also presented in study, but not summarized here:</u> arsenic levels in adipose, skeletal muscle, pancreas and liver; liver weight and water consumption</p>	5	[~ 4.7] As(III)	no effect
	5	[~ 6.25] As(III)	<p>impaired glucose tolerance following ip glucose challenge (IPGTT; ↑ peak blood glucose level, ~1.5-fold, at 15 min; ↑ blood glucose at 15, 30 and 60 minutes)</p> <p><u>Not affected at any dose level:</u> body weight; fasting blood glucose</p>
<p>(Paul et al. 2008) C57BL/6 mice (male); 0, 1, 10, 25, or 50 ppm sodium arsenite, As(III) in drinking water for 8 weeks. [~0.225, 2.25, 4.75, 6.25 mg/kg bw/day based on drinking water consumption reported by authors of 0.225 L/kg bw/d for 1 and 10 ppm, 0.19 L/kg bw/d for 25 ppm and 0.125 L/kg bw/d for 50 ppm] <u>Also presented in study, but not summarized here:</u> arsenic levels in adipose, skeletal muscle, pancreas and liver; liver weight and water consumption</p>	5	[~0.23] As(III)	no effect
	5	[~2.25] As(III)	no effect
	5	[~4.75] As(III)	no effect
	5	[~6.25] As(III)	<p>impaired glucose tolerance following ip glucose challenge (IPGTT; ↑ blood glucose at 15, 30 and 60 minutes)</p> <p><u>Not affected at any dose level:</u> body weight, fasting glucose</p>

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>(Paul <i>et al.</i> 2008) C57BL/6 mice (male); 0, 0.1, 1, 2.5, and 6 ppm methylarsine oxide, MAs(III) oxide, in drinking water for 8 weeks. [~0.0225, 0.225, 0.563, and 1.35 mg /kg bw/day based on drinking water consumption reported by authors of 0.225 L/kg bw/d] <u>Also presented in study, but not summarized here:</u> arsenic levels in adipose, skeletal muscle, pancreas and liver; liver weight and water consumption</p>	5/group	[~0.0225 – 1.35] MAs(III) oxide	<u>Not affected at any dose level:</u> body weight, fasting glucose, IPGTT
<p>(Paul <i>et al.</i> 2010) C57BL/6 mice (weanling male); 0, 25 (~57 µg/d), or 50 ppm (~81 µg/day) As(III) in drinking water for 20 weeks while consuming a high fat diet (HF diet, 58% fat) or low fat diet (LF diet, 11% fat)</p>		0	<p>HF diet vs. LF diet: ↑ body weight; ↑ fat mass; ↑ HOMA-IR; ↓ insulin sensitivity index; ↑ fasting blood glucose; ↑ serum insulin; ↑ liver triacylglycerol (TAG) *animals fed the LF diet had ↑ food consumption</p>
		25 ppm As(III)	HF diet vs. LF diet at 25 ppm: ↑ glucose intolerance
		25 ppm As(III)	25 ppm vs 0 ppm: ↓ water consumption
		50 ppm As(III)	HF diet vs. LF diet at 50 ppm: ↑ glucose intolerance
		50 ppm As(III)	<p>50 ppm vs 0 ppm: ↓ water consumption</p> <p>↓ body weight, fat mass, liver and serum TAG in HF diet group compared to HF diet controls</p> <p><u>Not affected in any group:</u> hematocrit (used as indicator of dehydration); β-cell function</p>
<p>(Reichl <i>et al.</i> 1990) NMRI mice (male), 0 or 12.9 mg/kg bw arsenic trioxide, As(III) oxide, by subcutaneous injection (single injection). Other animals were also treated with saline, 5% glucose or 0.12 IE insulin/kg by ip injection every 2 hours following As(III) oxide treatment and observed for 52 hours</p>	10	12.9 As(III) oxide	All animals in As(III) oxide-only group died within 22 hours (liver glucose and glycogen levels were ↓ in animals that died). ↑ Survival rate for As(III) oxide-treated animals subsequently treated with glucose (60% survival) or glucose + insulin (70 % survival).

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<p>(Singh and Rana 2009) Wistar rats (male), 0 or 4 mg/100g bw [40 mg/kg bw] arsenic trioxide, As(III) oxide, on alternating days for 30 days by [ip] injection; route of injection specified in (Singh <i>et al.</i> 2006). Primary purpose of study was to assess the effects of arsenic in diabetic rats (induced by treatment with 125 mg/kg bw alloxan by injection). Groups of diabetic rats were also treated with As(III) oxide, insulin, or As(III) oxide + insulin <u>Also presented in study, but not summarized here:</u> liver histopathology, liver arsenic levels, liver glucose-6-phosphatase, serum ALT, AST, bilirubin</p>	[Not stated]	40 As(III) oxide	<p>↑ glucose (~1.8 fold); ↓ insulin (~80% of control); ↓ body weight As(III) oxide treatment in diabetic rats caused a ↓ glucose and ↑ insulin. These findings plus effects on liver parameters led authors to conclude that diabetes can alter the pharmacodynamics and pharmacokinetics of arsenic, e.g., liver arsenic levels were reduced in diabetic animals treated with arsenic compared to the non-diabetic animals (853 µg/g versus 305 µg/g liver). Liver effects of As(III) oxide in diabetic rats were overall considered “protective”</p>
<p>(Wang <i>et al.</i> 2009) Sprague-Dawley rats (male); 0, 5, 15 or 30 mg/L “sodium arsenic” [assumed to be sodium arsenite, As(III)] in drinking water for up to 8 months (242 days) [~0.0475, 0.143, and 0.285 mg/kg bw/d based on drinking water consumption of 0.095 L/kg bw/d]. Arsenic effects on blood glucose were also assessed in animals that were treated with streptozotocin (STZ) to induce diabetes <u>Also presented in study, but not summarized here:</u> Urinary arsenic and N-acetyl-β-glucosaminidase (NAG)</p>	5-8	[~ 0.0475] As(III)	<p>altered glucose tolerance test at 6 months (↓ glucose levels at 15, 30, and 60 minutes in all dose groups) and at 8 months (↑ glucose levels at 15, 30, and 60 minutes); “restrained” the degree of STZ-induced diabetes condition by causing reductions in blood glucose</p>
	5-8	[~ 0.143] As(III)	<p>altered glucose tolerance test at 6 months (↓ glucose levels at 15, 30, and 60 minutes in all dose groups), but no effect after 8 months of treatment); “restrained” the degree of STZ-induced diabetes condition by causing reductions in blood glucose</p>
	5-8	[~0.285] As(III)	<p>altered glucose tolerance test at 6 months (↓ glucose levels at 15, 30, and 60 minutes in all dose groups), but no effect after 8 months of treatment); “restrained” the degree of STZ-induced diabetes condition by causing reductions in blood glucose <u>Not affected at any dose level:</u> blood glucose at anytime point – 140, 155, 170, and 210 days (not specified whether blood collected after fasting or ad libitum feeding)</p>
<p>(Yen <i>et al.</i> 2007) CD-1 (ICR) mice (male); 0 or 10 mg/L arsenic trioxide, As(III) oxide, in drinking water for 12 weeks [authors state that 5.4 ml/day was average amount of water consumed, average body weight of 20 g, water consumption of [~0.27 L/kg bw/d] <u>Also presented in study, but not summarized here:</u> This study also evaluated the effects of humic acid (HA) and HA + As(III) oxide on insulin and the pancreas histology. Results of HA treatment groups not summarized here. <i>In</i></p>	14	[~ 2.7] As(III) oxide	<p>↓ plasma insulin at 5, 7, and 12 weeks (~50% of control, unclear whether effect at 12 weeks was statistically significant); pancreatic effects of mild accumulation of inflammatory cells into the periductal and interstitial areas (reported as subacute peri-pancreatitis and pancreatitis); acinar cell atrophy loss; hyperplastic pancreatic islet cells in some slices</p>

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Appendix Table B. Summaries of arsenic studies in experimental animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects
<i>in vitro</i> studies described in			

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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
sodium arsenite	insulin secretion (GSIS)	isolated rat pancreatic β -cells	<p>↓ Viability after 72-hour incubation in pancreatic β-cells isolated from adult Wistar rats (10 μM LOEC) or 144-hour incubation</p> <hr/> <p>↓ basal insulin secretion in presence of 5.6 mM glucose after 144-hours or 15.6 mM glucose after 72-hours ; ↓ insulin mRNA expression in cells after 72-hour incubation</p>	<p>0, 0.5, 1, 2, 5*, and 10*</p> <hr/> <p>0, 1, 5*</p>	Diaz-Villasenor et al. (2006)
sodium arsenite	insulin secretion (GSIS)	RINm5F rat pancreatic β -cells	<p>↓ Viability ($\geq 1 \mu\text{M}$); ↓ basal and glucose stimulated insulin secretion ($\geq 1 \mu\text{M}$) [no effect on insulin mRNA or protein expression]; abates free $[\text{Ca}^{2+}]_i$ oscillations in response to glucose; ↑ glucose stimulated calpain activity (2 μM); inconsistent effects on SNAP-25 proteolysis; altered percentage of cells in G1, S, and G2/M phases, interpreted as arrest in the G2/M checkpoint ($\geq 2 \mu\text{M}$); ↓ mitotic index and replication index ($\geq 1 \mu\text{M}$)</p>	0, 0.5, 1*, 2*, and 2*	Diaz-Villasenor et al. (2008)
sodium arsenite	insulin secretion (GSIS)	INS-1 (832/13) rat pancreatic β -cells	<p>↓ insulin secretion with 3 or 20 mM glucose, GSIS (0.25-0.5 μM); ↑ insulin mRNA (0.1-0.5 μM) and content (0.25-0.5 μM); ↑ Nrf2 activity (0.25-0.5 μM), a transcription factor that regulates expression of many antioxidant/detoxification enzymes; ↑ expression of Nrf2-target genes (0.05-0.5 μM, depending on gene); ↑ cellular signs of oxidative stress (↑ intracellular GSH and H_2O_2-scavaging activity) (0.25-0.5 μM); ↓ glucose-stimulated peroxide production (0.25-0.5 μM); ↑ mitochondrial mass (0.25-0.5 μM); no effect of arsenite on classis GSIS pathways, i.e. expression of Glut2, Gck, K_{ATP}, and glucose-stimulated ATP production. [authors overall conclusions was that arsenite can activate Nrf2-mediated antioxidant response which can decrease ROS signaling involved in GSIS and disturb β-cell function]</p>	0, 0.05*, 0.1*, 0.25*, 0.5*	Fu et al. (2010)
sodium arsenite	mitochondrial substrate supply	isolated synaptosomes from guinea-pig cerebral cortex	<p>Arsenite 1 mM [1000 μM] used as an inhibitor of pyruvate dehydrogenase in a study looking at bioenergetic interactions between glycolysis and oxidative phosphorylation in isolated synaptosomes from guinea-pig cerebral cortex. ↓ Release of $^{14}\text{CO}_2$ from $[\text{I-}^{14}\text{C}]$pyruvate (i.e., inhibition of pyruvate oxidation); ↑ synaptosomal glycolysis in presence of glucose (3-fold); ↓</p>	1000* (cytotoxic)	Kauppinen et al. (1986)

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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
			synaptosomal respiration in presence of glucose. No effect on ATP, ADP, ATP+ADP, or ATP/ADP content in synaptosomes in presence of glucose		
oxophenylarsine (PhAsO)	glucose uptake	Madin-Darby canine kidney (MDCK) cells	<p>↓ Glucose uptake in MDCK cells after treatment with $2\mu\text{M}$ PhAsO (~40–50% of control values) [greater inhibition was observed at higher concentrations, $\geq 5\mu\text{M}$, but was accompanied by decreases in cell viability; glucose uptake in MDCK cells characterized as insulin-independent]. Several sulfur compounds reversed the inhibition of glucose uptake: 2,3-dimercapto-1-propanol (BAL), 2,3-dimercaptopropane-1-sulfonate sodium (DMPS), meso-2,3-dimercaptosuccinic acid (DMSA) and reversal was accompanied by decreased in cell-associated radiolabel from $[\text{U-}^{14}\text{C}]$-PhAsO. Other sulfur compounds had no/less of an effect or only an effect at high concentrations (100–200 μM): 2,3-bis(acetylthio)propanesulfonamide, dithiothreitol (DDT), 2-mercaptoethanol (ME) [authors overall conclusions were that the vicinal dithiols were most effective attenuating PhAsO inhibition of insulin-independent glucose uptake]</p>	0, 1, 2*, 5*, and 10*	Liebl et al. (1995a; 1995c)
oxophenylarsine (PhAsO)		MDCK cells	Overall purpose was to assess effects on PhAsO in “low” glucose (0.01 mmol/l) and “normal” glucose (5mmol/l) concentrations: ↓ glucose uptake at a “low” glucose concentration ($\text{IC}_{50} = 5 \times 10^{-5}$ mol/l PhAsO, 10 μM) and a “normal” glucose concentration ($\text{IC}_{50} = 2 \times 10^{-4}$ mol/l PhAsO, 5000 μM); ↓ cell viability at $\geq 5 \mu\text{M}$ PhAsO (based on formazan formation; greater impact in “low” glucose group); ↓ cellular ATP levels at “low” glucose, but no effect at the “normal” glucose concentration. Authors conclude that different uptake mechanisms operate at low and higher glucose states and that glucose can attenuate PhAsO-induced toxicity, i.e., reduced cell viability, cellular ATP, and glucose uptake	1, 2, 5*, 10*, 20*, 50*, 100*	Liebl et al. (1995b)
sodium arsenite	glucose-stimulated signal transduction	isolated islet from humans	Sodium arsenite ↑ binding of IUF1 to DNA (the same effect was caused by glucose alone), this effect was attenuated by treatment with the SAPK2 inhibitor SB 203580 but not wortmannin or LY 294002, which are 2 structurally unrelated inhibitors of phosphatidylinositide (PI) 3-kinase. All 3 of these inhibited glucose-	1000* (cytotoxic)	Macfarlane et al. (1997)

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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
sodium arsenite	glucose-stimulated signal transduction	MIN6, mouse β -cell line	stimulated IUF1 DNA-binding. \uparrow MAPKAP-K2 activity; \uparrow activity of human insulin promoter (pGL-LUC200) in transfected cells; These same effects were caused by glucose alone. The effects of sodium arsenite were attenuated by treatment with the SAPK2 inhibitor SB 203580 but not wortmannin or LY 294002. All 3 of these inhibited glucose-stimulated effects		
			Overall goal of study was to identify signal transduction pathways that mediate glucose-induced binding of IUF1 to DNA. UF1 is a β -cell specific transcription factor. Sodium arsenite was used as a “cellular stressor” to induce the activation of SAPK2 and MAPKAP-K2, a downstream target of SAPK2		
sodium arsenite	glucose-stimulated signal transduction	isolated islet from humans	\uparrow Activation of PDX1 and translocation to nucleus (the same effect was induced by glucose) after 30 minute incubation, this effect of sodium arsenite was attenuated by treatment with the SAPK2 inhibitor SB 203580 [PDX1 is involved in glucose-stimulated insulin gene transcription]	1000*	Macfarlane et al. (1999)
arsenate	insulin release and islet respiration	isolated islets from ob/ob mice	\downarrow glucose stimulated insulin release, \downarrow oxygen tension (at 11 mM glucose, but not in the basal condition of 3 mM glucose). Author’s conclusion is that arsenate uncoupled the reaction catalyzed by phosphoglycerate kinase from ATP production, resulting in reduced glycolytically produced ATP with no effect on glycolytic flux.	5000* (cytotoxic)	Ortsater et al. (2002)
arsenite (iAs ^{III})	glucose uptake/adipocyte function	3T3-L1 adipocytes	\downarrow insulin-stimulated glucose uptake after 4-h incubation ($\geq 5 \mu\text{M}$; ~20-70% of control) A follow-up study to assess the signal transduction pathways involved \downarrow insulin-dependent phosphorylation of PKB/Akt, which was implicated as a mechanism for \downarrow glucose uptake in 3T3-L1 adipocytes in Walton <i>et al.</i> , 2004 (Walton <i>et al.</i> 2004). \downarrow PDK-1 activity (50 μM); PDK-1 shown to catalyze PKB/Akt phosphorylation and PKB/Akt activity was \downarrow in exposed cells. PI-3K and PTEN activities were considered unaffected.	0.5, 5*, 10*, 25*, 50*, 100*, 500*, 5000*	Paul et al. (2007a)
methylarsine oxide (MAs ^{III} O)	glucose uptake	3T3-L1 adipocytes	\downarrow insulin-stimulated glucose uptake after 4-h incubation ($\geq 0.5 \mu\text{M}$; ~10-85% of control)	0, 0.5*, 1*, 2*, 5*, 10*, and 20*	Paul et al. (2007a)

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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
			A follow-up study to assess the signal transduction pathways involved \downarrow insulin-dependent phosphorylation of PKB/Akt, which was implicated as a mechanism for \downarrow glucose uptake in 3T3-L1 adipocytes in Walton <i>et al.</i> , 2004 (Walton <i>et al.</i> 2004). \downarrow PDK-1 activity (2 μM); PDK-1 shown to catalyze PKB/Akt phosphorylation and PKB/Akt activity was \downarrow in exposed cells. PI-3K and PTEN activities were considered unaffected.		
sodium arsenate	glucose uptake	rat liver Clone 9 cells	Arsenic used as a "mitochondrial poison" to stimulated sugar (2-deoxyglucose) uptake [used as an indicator of GLUT1 activity in liver cells], this effect was blocked by co-treatment with bapta, a membrane permeable calcium chelator	200* (cytotoxic)	Quintanilla et al. (2000)
Arsenic trioxide (As ₂ O ₃)	adipocyte differentiation	3T3-F442A pre-adipocytes	Treatment with sub-toxic concentrations for 3-days induced the expression of genes involved in adipose differentiation (PPAR γ and C/EBP α (0.5 μM with or without insulin), oxidative stress response (HO1, HIF1 α (0.25 μM in presence of insulin)) and cell-cycle regulation (KLF5 (0.25 μM in absence of insulin), but not c-jun). No changes in morphology or lipid droplet accumulation were observed in treated adipocytes up to 15-17 days culture; inhibition of adipogenesis and decreased cell viability found at longer times of exposure	0.005, 0.05, 0.25*, 0.5*, 1*, 5*, 15*, 25*	Salazard et al. (2004)
phenylarsine oxide (PAO)	glucose uptake	L929 fibroblast	Measured glucose uptake in L929 fibroblast cells which only express GLUT1. \uparrow glucose uptake at low concentrations of PAO (1-5 μM , up to \sim 4-fold) and \downarrow glucose uptake at a high concentration (40 μM , \sim 35% of control value); \downarrow glucose uptake induced by glucose deprivation (\geq 2 μM) and methylene blue (\geq 5 μM)	0, 0.5, 1, 2*, 5*, 10*, 20*, 30*, and 40*	Scott et al. (2009)
arsenic trioxide (As ₂ O ₃)	gluconeogenesis	isolated rat hepatocytes or kidney tubules	\downarrow gluconeogenesis following treatment with a variety of substrates (pyruvate (90% reduction at 0.05 μM , 30 min) > malate > ketoglutarate > succinate > fructose > dihydroxyacetone (40% reduction at 0.05 μM , 30 min)) in rat kidney suspensions. Similar results were observed with hepatocyte suspensions (data not shown). To a lesser extent \downarrow O ₂ consumption and \downarrow ATP content were observed (both tissues). \downarrow Content of acetyl-CoA and glutathione (rat kidney tubules suspension) or 3-hydroxybutyrate	0, 0.005*, 0.01*, 0.02*, 0.025*, 0.05*	Szinicz et al. (1988)

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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
			(hepatocyte suspensions) incubated with As_2O_3 . Authors overall conclusions were that inhibition of pyruvate dehydrogenase is a central target of these arsenicals		
arsenic pentaoxide (As_2O_5)	gluconeogenesis	isolated rat kidney tubules	\downarrow gluconeogenesis following treatment with a variety of substrates (pyruvate (90% reduction at 1 μM , 30 min) > succinate > ketoglutarate > lactate > fructose > malate > dihydroxyacetone (60% reduction at 1 μM , 30 min)). To a lesser extent \downarrow O_2 consumption and \downarrow ATP content were observed. Authors noted that compared to As_2O_3 about 5 to 10-times higher concentrations of As_2O_5 were needed to induce similar amounts of \downarrow gluconeogenesis, \downarrow O_2 consumption, and \downarrow ATP content. \downarrow Authors overall conclusions were that inhibition of pyruvate dehydrogenase is a central target of these arsenicals	0, 0.1*, 0.25*, 0.5*, and 1*	Szinicz et al. (1988)
Sodium arsenite	adipocyte differentiation	C3H 10T1/2 preadipocytes	\downarrow differentiation as reflected by cellular lipid content when cells exposed to As (6 μM , 8 wks) prior to induction (dex/insulin); As need not be continued after induction for this effect. Dose-dependent \downarrow differentiation observed when As (3, 6, and 10 μM) added during the differentiation protocol. Added to differentiated cells (6 μM) results in lipid vesicle fragmentation and/or shrinkage. A second set of experiments focused on the impact of As on proliferation. \uparrow in % of cells in S phase (~3.5 fold increase), cell proliferation, thymidine incorporation observed in cells maintained in As (6 μM) through differentiation, but removed prior to measuring endpoints, suggesting cells cultured in As acquire an altered mitogenic potential that is masked by continued exposure.	0, 0.1, 0.3, 1, 3*, 6*, and 10*	Trouba et al. (2000)
arsenite (iAs^{III})	glucose uptake/adipocyte function	3T3-L1 adipocytes	\downarrow insulin-stimulated glucose uptake in 3T3-L1 adipocytes after 24-hour incubation (≥ 10 μM , ~20-48% of control); \downarrow basal glucose after 4-h incubation (100 μM , ~62% of control); cytotoxicity (50 μM after 24-h incubation, 100 μM after 4-h incubation); \downarrow immunoblot detection of PKB/Akt at 50 μM after 4-h incubation (no effect on IR β phosphorylation, IRS-1 or IRS-2 phosphorylation, PI-3K); \downarrow immunofluorescence of insulin-responsive GLUT4 in cell membrane at 50 μM after 4-h incubation. <i>Cell and medium concentrations</i> of iAs^{III} and its methylated metabolites measured: 21% of iAs^{III}	5, 10*, 20*, 50*, and 100*	Walton et al. (2004)

Appendix Tables
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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
			associated with cells, methylarsine and iododimethylarsine metabolites found in cell compartment, but not medium.		
methylarsine oxide ($\text{MAs}^{\text{III}}\text{O}$)	glucose uptake/adipocyte function	3T3-L1 adipocytes	\downarrow insulin-stimulated glucose uptake in 3T3-L1 adipocytes after 24-hour incubation (1 μM , ~50% of control); \downarrow basal glucose after 4-h incubation (5 μM , ~40% of control); cytotoxicity (2 μM after 24-h incubation, 5 μM after 4-h incubation); \downarrow immunoblot detection of PKB/Akt and \uparrow IRS-1 phosphorylation at 2 μM after 4-h incubation (no effect on IR β phosphorylation, IRS-2 phosphorylation, PI-3K); \downarrow immunofluorescence of insulin-responsive GLUT4 in cell membrane at 2 μM after 4-h incubation.	0.25, 0.5, 1, 2*, and 5*	Walton et al. (2004)
iodoimethylarsine oxide ($\text{DMA}^{\text{III}}\text{O}$)	glucose uptake/adipocyte function	3T3-L1 adipocytes	\downarrow insulin-stimulated glucose uptake in 3T3-L1 adipocytes after 4-hour incubation ($\geq 2 \mu\text{M}$, ~20-55% of control; no effect with 24-h incubation at 0.5-2 μM); no effect on basal glucose after 4-h incubation (tested at 2 and 10 μM); cytotoxicity (5 μM after 24-h incubation, 10 μM after 4-h incubation); \downarrow immunoblot detection of PKB/Akt, \downarrow IR β phosphorylation, and \uparrow IRS-1 phosphorylation at 5 μM after 4-h incubation (no effect on IRS-2 phosphorylation, PI-3K); \downarrow immunofluorescence of insulin-responsive GLUT4 in cell membrane at 5 μM after 4-h incubation.	1, 2*, 5*, and 10*	Walton et al. (2004)
arsenate (iAs^{V})	glucose uptake/adipocyte function	3T3-L1 adipocytes	\uparrow basal glucose uptake in 3T3-L1 adipocytes after 4-h incubation at 100 μM (~1.45-fold); \downarrow in basal glucose uptake after 4-h incubation at 1000 μM (~70% of control); no effect on insulin-stimulated glucose uptake after 4-hour incubation (100-1000 μM)	100* and 1000*	Walton et al. (2004)
methylarsonate same as methylarsenic acid (sodium salt) (MAs^{V})	glucose uptake/adipocyte function	3T3-L1 adipocytes	\downarrow insulin-stimulated glucose in 3T3-L1 adipocytes after 4-h incubation at $\geq 100 \mu\text{M}$ (~80% of control); no effect on basal glucose uptake after 4-hour incubation (100-1000 μM)	100* and 1000*	Walton et al. (2004)
dimethylarsinic acid (DMA^{V})	glucose uptake/adipocyte function	3T3-L1 adipocytes	no effect on basal or insulin-stimulated glucose uptake in 3T3-L1 adipocytes after 4-hour incubation (100-1000 μM)	no effect (tested up to 1000 μM)	Walton et al. (2004)
sodium arsenite	expression of fat-specific genes (aP2, PPAR γ , and	C3H 10T1/2 cells	\downarrow aP2, PPAR γ , and C/EBP α mRNA levels (6 μM) under all tested culture conditions in C3H 10T1/2 cells (pluripotent cell line that can be induced to differentiate into adipocytes) (As added at the start of	6*	Wauson et al. (2002)

Appendix Tables
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Appendix Table C. Summary of arsenic *in vitro*/mechanistic studies

Chemical	Effect	Cell/Tissue	Mechanistic Effect	Concentration range tested (μM)	Reference
	C/EBP α)		differentiation, treated long term but switched to As-free medium at start of differentiation, or cell treated continuously with As before and after start of differentiation). As (6 μM) prevented pioglitazone-induced differentiation. Inhibition of MEK-1/2 with U0126 did not block As inhibition of insulin/dexamethasone- or pioglitazone-induced adipogenesis.		
phenylarsine oxide (PAO)	glucose uptake/transporter	BHK cells	Reported to alter the plasma membrane-perinuclear region distribution of glucose transporter (visualized by Hep G2 antibody) in insulin-treated baby hamster kidney (BHK) cells; no effect in cells not treated with insulin	35* (cytotoxic)	Widnell et al. (1990)
sodium arsenite	glucose uptake/transporter	BHK cells	Reported to alter the plasma membrane-perinuclear region distribution of glucose transporter (visualized by Hep G2 antibody) in BHK cells	200* (cytotoxic)	Widnell et al. (1990)
arsenic trioxide (As ₂ O ₃)	pancreatic β cell viability and function	HIT-T15 hamster β -cells	Treatment of hamster β pancreatic (HIT-T15) with arsenic caused \downarrow cell viability at 1-10 μM (~20-80% of control), apoptosis (assessed at 2.5 μM), ATP depletion at 2.5-10 μM (~20-80%) of control after 24-hour treatment, oxidative stress based on \uparrow reactive oxygen ROS content at 5-20 μM , \uparrow caspase 3 activity (assessed at 2.5 μM , 4-fold), and altered \downarrow insulin cell content at 5 μM (but not 1 or 2.5 μM)	0, 1*, 2.5*, 5*, 7.5*, 10*	Yen et al. (2007)

\uparrow , \downarrow = Statistically significant increase or decrease

Abbreviations: baby hamster kidney cells (BHK); β -subunits of the insulin receptor (IR β); insulin receptor substrate (IRS); mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP-K2); transcription factor NF-E2-related factor 2 (Nrf2); phosphatidylinositol-3 kinase (PI-3K); pancreatic/duodenal homeobox-1 (PDX1); phosphatase and tensin homolog deleted on chromosome ten (PTEN); protein kinase B (PKB/Akt); reactive oxygen species (ROS); stress-activated protein kinase 2 (SAPK2); synaptosomal-associated protein of 25 kDa (SNAP-25) Peroxisome proliferator-activated receptor γ (PPAR γ); CCAAT/enhancer binding protein (C/EBP α), oxidative stress response heme-oxygenase-1 (HO1); hypoxia inducible factor 1a (HIF1 α); Kruppel like factor 5 (KLF5)

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