MODELING THE EFFECTS OF DIETARY ARSENIC AND NUTRIENT INTAKE ON URINARY ARSENIC BIOMARKERS

by

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ABSTRACT

Background: Arsenic (As) is a naturally-occurring element with known toxicant effects. The primary exposure pathway is through ingestion, but the overall contribution of food versus water and the impact of specific dietary nutrients on urinary As excretion is not well understood.

Methods: Secondary analyses of laboratory results from food, water and urine samples, questionnaire and anthropometric data, and dietary records were performed on four study populations: the National Health Exposure Assessment Survey (NHEXAS)-Arizona, Arizona Border Survey (ABS), the Arizona sub-group of the Binational Arsenic Exposure Survey (BAsES), and the 2003-2004 National Health and Nutrition Examination Survey (NHANES). Dietary As intake was measured in duplicate food samples and/or modeled from dietary records for each population using the U.S. Total Diet Study (TDS) arsenic residue database and a published market basket survey. Urinary total As, As^5, As^3, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were analyzed, and sum of species As was calculated as the sum of As^5, As^3, MMA and DMA. Regression analyses

modeled the relation between urinary As biomarkers (total, sum of species, MMA:sum of species, and DMA:MMA) and dietary As, adjusted for drinking and cooking water As intake, current smoking, sex, age, ethnicity, body mass index, and nutrient intake.

Results: Modeled dietary As based on TDS mean As residue data greatly underestimated exposure as compared with measured As in duplicate diet samples and estimates based on other residue data. Dietary As was a significant predictor of urinary total As in all four populations, of sum of species As in both BAsES and NHANES, and of %MMA and DMA:MMA in NHANES. Dietary protein intake was associated with decreased sum of species As in both BAsES and NHANES, but dietary folate was not.

Conclusions: Dietary As contributes a markedly greater proportion of total ingested As and is a better predictor of urinary As than water As intake in the U.S. Among subjects who did not consume seafood, total As exposure from food and water exceeded the provisional tolerable daily intake of 2.1 µg/kg body weight/day in 3-15% of these study populations. Increased protein intake may mitigate the effects of As.

INTRODUCTION

Arsenic (As) is a metalloid element with known toxicant and carcinogenic effects. Ingestion is the primary exposure pathway (O'Rourke et al., 1999a; Hughes, 2006; Pellizzari and Clayton, 2006). Arsenic--predominantly inorganic--enters the food chain through naturally-occurring contamination of groundwater and, to a lesser extent, through industrial means (i.e., agricultural pesticide and herbicide use, use as a poultry feed supplement, soil and water contamination from mining and smelting ore) (Garelick et al., 2008). In the U.S., increased groundwater concentrations are primarily found in or near mining and smelting communities located in heavily mineralized areas (Garelick et al., 2008). Water from rural (O'Rourke et al., 1999b; Smedley and Kinniburgh, 2002) and Native American communities (Navas-Acien et al., 2009) may be particularly affected. It has been estimated that high concentrations of As in groundwater pose a public health risk to approximately 13 million people in the U.S., and 57 million people worldwide (Chen, Y. et al., 2011).

In 2001, the U.S. Environmental Protection Agency (EPA)

reduced the maximum contaminant level (MCL) for As in drinking water from 50 µg/l (50 ppb) to 10 µg/l (10 ppb), with compliance to the new standard required of all municipal water providers by 2006 (EPA, 2010). Recent studies in the U.S. indicate, however, that food, rather than drinking water, provides the majority of exposure to arsenic in non-occupational settings (Hughes, 2006), and that dietary exposure may be as much as 14 times greater than that from drinking water (Xue et al., 2010).

Assessing the role of food is complicated by the heterogeneity of food sources, as well as by the concentration of As in soils and in water used for irrigation and food preparation. Foods comprise a complex matrix and contain various arsenical compounds that make laboratory analysis challenging (Meharg and Raab, 2010). Existing food As residue data is highly variable, and recent studies (Meharg et al., 2008; Roberge, J. et al., 2009; Jackson et al., 2012) suggest that the U.S. Food and Drug Administration (FDA) databases may underestimate risk. There are currently no standards for arsenic in most food products and few studies have investigated the contribution of food to total As exposure. Furthermore, the relation between dietary As and nutrient intake and human As

metabolism is not well understood.

Metabolism of As involves the methylation of inorganic forms of As into monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) and is generally considered a process of detoxification. There is variation among individuals in their ability to methylate inorganic As, as evidenced by differences in the proportions of urinary As biomarkers excreted. Arsenic methylation incorporates one-carbon metabolism, which utilizes specific nutrients, including folate, vitamin B_{12}, vitamin B_6, methionine and protein, to facilitate methylation. The efficiency with which As is methylated and excreted may be attributable, in part, to the availability of these nutrients in the diet.

The overall objectives of this research were to evaluate the contribution of dietary arsenic intake to urinary arsenic biomarkers in four distinct study populations, both regional and national, and to assess the effects of ethnicity, dietary nutrient intake, and household tap water As concentration on these biomarkers.

This dissertation utilizes data from three regional and one national study population, all well-characterized for exposure to arsenic: 1)National Health Exposure Assessment Survey (NHEXAS)-Arizona, 2)Arizona Border Survey (ABS),

3)the Arizona sub-group of the Binational Arsenic Exposure Survey (BAsES), and 4)2003-2004 National Health and Nutrition Examination Survey (NHANES). Based on collection and analysis of dietary data, first morning void urine samples, water samples, and questionnaire data, the relationship between dietary As and nutrient intake and urinary As excretion is compared by study population, ethnicity, and household water As concentration.

SPECIFIC AIMS AND HYPOTHESES

Data from the four population studies were used to address a series of specific questions. The following specific aims and hypotheses were addressed in this dissertation:

Specific Aim 1: To compare measured versus modeled estimates of total dietary arsenic as predictors of total urinary arsenic in the National Human Exposure Assessment Survey (NHEXAS)-AZ and Arizona Border Survey (ABS) populations. Dietary arsenic intake was measured from 24-hour duplicate diet samples and modeled based on 24-hour diet diaries and published arsenic residue data from: 1) the U.S. FDA Total Diet Study (TDS) Statistics on Element Results (1991-2003) (F.D.A., 2007) and 2) a limited market basket survey of food commodities assumed to contribute 90% of dietary inorganic As (Schoof et al., 1999)

Hypothesis 1a: Modeled estimates of dietary total As will yield estimates of As intake that are comparable to

total As measured in the duplicate diet samples from the same day of record.

Hypothesis 1b: Regression models of urinary total arsenic and dietary total arsenic measured from 24-hour duplicate diet samples will provide a better fit to the data than models using estimates of dietary As intake based on As food residue databases and food diaries.

Specific Aim 2: To evaluate the contribution of modeled dietary total and inorganic arsenic and water arsenic on total and speciated urinary arsenic in Hispanic and non-Hispanic Arizonans in the Binational Arsenic Exposure Study (BAsES). The effects of dietary nutrient intake on arsenic methylation and effect modification by exposure to household water As concentrations above versus below the MCL (10 ppb) will be evaluated.

Hypothesis 2a: Modeled estimates of dietary arsenic intake will explain a greater extent of urinary As excretion (total and sum of species) than As intake from drinking and cooking water, especially in households with tap water concentrations ≤ 10 ppb.

Hypothesis 2b: Hispanic whites in the population will ingest higher levels of dietary As and excrete greater concentrations of urinary total As and As sum of species than non-Hispanic whites.

Hypothesis 2c: Dietary micronutrients involved in the As methylation pathway, such as folate, protein, methionine, and vitamins B-6 and B-12, will modulate the effects of dietary arsenic exposure and will be associated with increased methylation efficiency (i.e., low percent MMA and high DMA:MMA ratio).

Specific Aim 3: To evaluate the contribution of modeled dietary total and inorganic arsenic intake and the effects of specific dietary nutrient intake on patterns of arsenic methylation in a nationally representative U.S. population sample from the 2003-2004 National Health and Nutrition Examination Survey (NHANES). The potential for differences in the effects among ethnic groups will be evaluated, and the national results will be contrasted with the regional results obtained in Aim 2.

Hypothesis 3a: Modeled dietary arsenic intake, based on 24-hour dietary recall interviews and published arsenic

residue data, will be statistically significant positive predictors of urinary total As and As sum of species.

Hypothesis 3b: Increased dietary folate, protein, and vitamins B-6 and B-12 will modulate the effects of dietary arsenic exposure and will be associated with increased methylation efficiency (i.e., low percent MMA and high DMA:MMA ratio).

Hypothesis 3c: The national results, despite the absence of data on exposure to arsenic in drinking and cooking water, will corroborate the results from the BAsES population.

BACKGROUND AND LITERATURE REVIEW

I. Environmental Sources of Arsenic

Arsenic (As) is the 20th most abundant element in the earth's crust (Yost et al., 1998). Typically found in geological materials at concentrations between one and five parts per million (ppm), it can occur at much greater concentrations (Spencer, 2002). Over 200 mineral species contain inorganic arsenic, in many different oxidation states (generally +3 and +5) and forms (Hughes, 2006). Arsenic is persistent in the environment, attaching to particles in the soil and dissolving in water (ATSDR, 2007). The names and formulas of the most common inorganic and organic arsenic compounds found in water, soil, and food, and referred to in this study are presented in Table 1.1.

TABLE 1.1. Common inorganic and organic arsenic compounds.

	synonyms	abbreviation	formula
Inorganic Arsenic			
Arsenate	pentavalent arsenic, inorganic arsenic^{V}, arsenic acid	As^{+5}	$AsO(OH_3)$
Arsenite	trivalent arsenic, inorganic arsenic^{III}, arsenous acid	As^{+3}	$As(OH_3)$
Organic Arsenic			
Monomethylarsonic acid	methylarsonic acid	MMA^{+5}	$CH_3AsO(OH)_2$
Monomethylarsonous acid	methylarsonous acid	MMA^{+3}	$CH_3As(OH)_2$
Dimethylarsinic acid		DMA^{+5}	$(CH_3)_2AsO(OH)$
Dimethylarsinous acid		DMA^{+3}	$(CH_3)_2As(OH)$
Arsenobetaine		AsB	$(CH_3)_3As + CH_2COOH$
Arsenocholine		AsC	$C_5H_{14}AsO^{+}$
Arsenosugars			$(CH_3)_2AsO_2H$

Inorganic arsenic generally occurs in two oxidation or valence states, as pentavalent arsenic or arsenate (As^{+5}) and as trivalent arsenic or arsenite (As^{+3}). Through metabolism, inorganic As is transformed into two organic methylated compounds, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which undergo further biotransformations (discussed in later sections). Fish and shellfish accumulate organic arsenic, primarily in the form of arsenobetaine (AsB), but also as arsenocholine (AsC) and arsenosugars (ATSDR, 2007).

A. Arsenic in soils

The content of naturally-occurring arsenic in soils worldwide generally ranges between 2000-20,000 ppb, but can exceed 600,000 ppb (Cohen et al., 2006). Certain geological substrates and geographic regions are associated with elevated inorganic As concentrations (Welch and Ryker, 2000), and ground water arsenic in these regions may be naturally elevated. Copper, zinc, gold and lead-bearing minerals are especially rich in inorganic As (Garelick et al., 2008), as are soils derived from volcanic eruptions. Surface runoff and percolating groundwater from springs with high arsenic concentrations can also increase the

arsenic content of the surrounding soils (Peryea, 1999). The relationship between plants and soil arsenic, arsenic solubility and mobility vary depending on the chemical form(s) of the element in the soil, climate, properties of the soil, agricultural management practices, and the plant species and varieties grown (Peryea, 1999).

B. Arsenic in water

The amount of arsenic in groundwater is dependent on the geology, hydrology and geochemistry of the aquifer sediments (Garelick et al., 2008). Complex geochemical processes--i.e., oxidation of arsenic-bearing sulfides, desorption and reductive dissolution of arsenic from oxides and hydrides, and leaching from sulfides (Garelick et al., 2008)--influence the amount of arsenic found in groundwater. According to Smedley and Kinniburgh (2002), there are two "triggers" that can induce large scale release of arsenic: 1) the combined effects of mineral weathering and high evaporation in high pH (>8.5) conditions in arid or semi-arid environments, and 2) strongly reducing conditions at approximately neutral pH causing the desorption, reduction, and dissolution of arsenic from mineral oxides. Surface and ground water

sources in mining districts or near smelters are at greatest risk of contamination (Smedley and Kinniburgh, 2002).

Arsenic concentrations in ground and surface waters are highly variable, ranging from less than 1 microgram per liter (µg/L) to over 5000 µg/L, but the average concentration is 1-2 µg/L (ATSDR, 2007). High concentrations occur naturally in diverse regions of the world, including Bangladesh, Bengal (India), Vietnam, China, Hungary, Romania, Argentina, Chile, Mexico, and several locations in the U.S. (Smedley and Kinniburgh, 2002; Garelick et al., 2008). In most areas where chronic arsenic poisoning occurs, arsenic exceeds 1000 µg/L (IPCS, 1981). In the U.S., only 2% of drinking water supplies exceed 20 µg As/L and 80% contain less than 2 µg/L (ATSDR, 2007; Chen, Y. et al., 2011). However, As concentrations up to 1700 µg/L have been measured in well water in Oregon (Focazio, 2000).

The concentration of As in unpolluted surface waters, such as streams, rivers and lakes, is typically <10 µg/L, though 800 µg/L has been reported (IPCS, 1981). Arsenic species prevalent in ground water are arsenate (As^{+5}), under acidic conditions, and arsenite (As^{+3}), at pH > 7 (Spencer,

2002). The ratio of As^{+3} to As^{+5} appears to be highly variable. In stream water samples, in the presence of dissolved oxygen, As^{+5} dominates and As^{+3} constitutes only 8% of the total arsenic, while in samples from deep wells, under reducing conditions, As^{+3} is dominant (IPCS, 1981; IARC, 2004).

Other arsenic species that occur in drinking water in minor amounts include MMA^{+5}, MMA^{+3}, DMA^{+5}, DMA^{+3}, and trimethylarsine oxide (TMAO). Additional arsenic species have been reported from some sources (IARC, 2004).

C. Arsenic uptake by plants

Plant arsenic concentrations tend to increase with increasing soil arsenic, but plants vary in their resistance to As toxicity (Peryea, 1999). Arsenate, the primary form of arsenic in soils, competes with phosphate for uptake by plant roots. In a review of studies on arsenic metabolism in plants, the role of soil microbes and mycorrhizal fungi in the arsenate uptake of plants is discussed (Meharg and Hartley-Whitaker, 2002). The presence, mostly in minor amounts, of methylated arsenic species in plants is mentioned, but it is unclear whether these compounds are converted by the plants to organic

forms or absorbed from solution in the soil (Meharg and Hartley-Whitaker, 2002). Some species of plants show considerable arsenic resistance, while other species exhibit growth inhibition or death. Genetic variation in arsenic uptake by plants has been observed (Meharg and Hartley-Whitaker, 2002).

II. Anthropogenic Sources of Arsenic

Historical uses of arsenic include medicinal and pharmaceutical products and pigment and glass manufacturing. Currently, arsenic is used in the production of: 1) agricultural pesticides in the form of monosodium methylarsonate, disodium methylarsonate, dimethylarsinic acid, and arsenic acid; 2) wood preservative salts, in the form of arsenic acid; and 3) arsenillic acid as an additive to poultry and other animal feeds (Garelick et al., 2008). Additional anthropogenic sources of arsenic include ore production and processing, high-temperature burning of oil and coal, solar cells, electronics, various pharmaceutical drugs (for the treatment of malaria, syphilis, leukemia and psoriasis)(Yoshida et al., 2004), household waste and incineration, ceramics and glassware production, and drying agents for cotton (Garelick et al., 2008).

For over 100 years, arsenic has been used in agricultural pesticides and insecticides. Orchards, in particular, have been treated with lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenate, zinc arsenite and copper-arsenic compounds, leading to arsenic contamination

of soils (Garelick et al., 2008). Arsenical pesticides were extensively applied to fruit orchards (apples and peaches), blueberries, potato crops, turf farms and golf courses up until about the 1960's (Members, 1999). They were used to control specific pests, such as the Colorado potato beetle and cotton boll weevil, and sprayed and dusted on fruits and vegetables as broad-spectrum insecticides. There are reports of point-source contamination at storage facilities (Smith, E. et al., 2003). In some fields, where there was widespread use of these insecticides, the As concentration in the soil is toxic to fruit trees (Members, 1999). In general, former fields are evaluated for arsenic contamination prior to conversion to other uses.

The timber industry has been using chromated copper arsenate (CCA)--which contains 22% pure arsenic--to prevent the decomposition of wood products due to wood-boring crustaceans, mollusks and fungi. In recent years the use of CCA-treated wood has been restricted, but disposal poses a hazard due to leaching (Garelick et al., 2008).

III. Human Exposure: Ingestion

A. Drinking water

Globally, an estimated 50-60 million people are chronically exposed to As in excess of 10 µg/L in their drinking water. In the U.S., the number of people at increased risk of arsenic-induced health effects from their drinking water is estimated at 13 million (Chen, Y. et al., 2011).

Standards have been established for arsenic contamination of potable water. The World Health Organization (WHO) set an arsenic standard of 10 µg/L of water (equivalent to 10 ppb) as a provisional, health-based guideline. This concentration is associated with an excess lifetime cancer risk of approximately 1/100,000 (Yamamura, 1998). In 2001, the U.S. Environmental Protection Agency (EPA) reduced the Maximum Contaminant Level (MCL) for arsenic in drinking water from 50 ppb to 10 ppb. Compliance with this new standard was required of all community water systems and nontransient, noncommunity water systems by January 22, 2006 (EPA, 2001; EPA, 2010). The U.S. Food and Drug Administration established an allowable level for arsenic in bottled water of 10 µg/L of water, the same limits that

apply to community drinking water supplies (FDA, 2011).

B. Foods

Arsenic is present in food in both organic and inorganic forms and is the largest source of arsenic exposure to people living in most parts of the U.S. (Chappell et al., 1997; ATSDR, 2007). Of the approximately 260 prepared foods analyzed in the Total Diet Study (TDS) (1991-96), arsenic was found in 24% (Tao and Bolger, 1999). Data on the chemical forms of arsenic in most foods is limited (Egan et al., 2002) and some of it is contradictory.

Aquatic organisms, both fish and shellfish, contain markedly higher concentrations of total arsenic than other foods (Lorenzana RM, 2009), up to several milligrams of arsenic per gram. Generally, more than 80% of the arsenic in seafood is in organic forms, primarily as arsenobetaine (ATSDR, 2007). Organic arsenic is generally perceived as having low toxicity (Meharg and Raab, 2010). However, some shellfish, even from uncontaminated waters, may contain as much as 30% inorganic arsenic (Lorenzana RM, 2009).

Rice is a staple food for much of the world's population. Market-purchased rice shows a large variation in the range of concentrations of total arsenic, from 0.04 to 0.28 mg/kg

(Cascio et al., 2011). A study of 204 commercial rice samples found differences in arsenic content between various types of rice, with brown and long grain rice containing higher mean arsenic concentrations than white rice (Zavala and Duxbury, 2008). Within the U.S., higher arsenic concentrations have been found in rice grown in the South Central U.S., as compared with rice grown in California (Williams et al., 2007). Overall, the mean concentration of arsenic in U.S. rice is similar to that grown in Europe (0.198 mg/kg), but tends to be higher in total arsenic than Asian rice (0.07 mg/kg) (Zavala and Duxbury, 2008).

A market basket study of rice purchased in the United Kingdom reported differences in total As and in the proportion of inorganic to total arsenic in rice imported from different countries. The proportion of inorganic As in rice from Italy and India was 58% and 56%, respectively, but the proportion of inorganic As in rice imported from the U.S. was only 37% (Cascio et al., 2011). The proportion of inorganic to total arsenic in rice varied from 16-49% in a U.S. survey, and these samples also had high mean concentrations of dimethylarsinic acid (DMA) (Schoof et al., 1999). Zavala et al. (2008) evaluated

arsenic species in 29 market basket rice samples from different regions (Zavala et al., 2008). The predominant arsenic species were arsenite (As^{+3}) and DMA. In this study, they identified two populations of rice on the basis of total arsenic and percent DMA. In samples with the highest total arsenic (mostly U.S.), DMA increased linearly with total arsenic and made up a larger proportion of the total arsenic. A second population of rice (Asian and European) was identified in which the dominant form of arsenic was inorganic (As^{+3} and As^{+5}). The authors hypothesized that the high DMA plants may have originated in fields in which arsenical pesticides had been previously applied to cotton. They further postulated that the rice developed arsenic resistance, an ability to metabolize and detoxify arsenic, and that the DMA rice type might be less toxic to humans (Zavala et al., 2008).

Recent studies provide a strong argument for the need to improve assessment of food and dietary arsenic exposure. In a study of commercial beverages purchased in Tucson, Arizona, extensive variability in the concentration of arsenic was reported, both between and within types and brands of food (Roberge et al., 2009). Furthermore, the proportion of arsenic species reported in most studies is

highly variable (Yost et al., 1998). Inorganic arsenic constitutes between 7-95% of total arsenic in the plant-based commodities measured in a market basket survey. In most of these foods (including rice, flour, grape juice, spinach, peanut butter and cucumber), the concentration of As^{+3} was generally twice that of As^{+5}. Beet and cane sugars had higher As^{+5} than As^{+3}. In general, DMA levels were low in foods, though measurable in rice, shellfish, beet and cane sugars, meat, fruits and fruit juices, and MMA was detectable only in apple juice (Schoof et al., 1999).

Studies of total, inorganic and organic arsenic concentrations in chicken purchased in North America also report extensive variability. Lasky estimates a mean intake of inorganic As from chicken consumption of 1.4-5.2 µg/day, based on ingestion of 60 g (2.1 ounces) of chicken per day (Lasky et al., 2004). Arsenic is a component of approved drugs or additives to poultry and other animal foods (Lasky et al., 2004). The commonly used additive Roxarsone contains organic As in the +5 oxidative state, and is excreted by chickens either unchanged or as an amino-metabolite. However, there appears to be little data on As residue compounds present in chicken meat (Lasky et al., 2004), and high variability in the proportion of

inorganic to organic As in chicken. In a 1999 market basket survey, only 1% of total As in chicken samples was inorganic (Schoof et al., 1999), though an earlier study reported that 41% of the total was inorganic (Yost et al., 1998). In a Canadian study, 65% of total arsenic in chicken samples was inorganic (Lasky et al., 2004).

C. Dietary arsenic intake

Specific food items, such as rice, produce and chicken, appear to comprise the greatest proportion of our dietary intake of inorganic As (Schoof et al., 1999; Tao and Bolger, 1999; Hughes, 2006; Cascio et al., 2011). In the NHEXAS-Maryland study, tuna and other fish, shrimp, and spinach were highly significant predictors of total As content of duplicate diet samples (Ryan et al., 2001). In a national population sample, food items were ranked based on their modeled contribution to dietary intake of inorganic As (MacIntosh et al., 1997) (see Table 3.1).

TABLE 3.1. The top 10 foods contributing to total dietary intake of inorganic arsenic (MacIntosh et al., 1997).

1.	white rice	14.95%
2.	shrimp	11.38%
3.	skinless chicken	9.97%
4.	brown rice	6.99%
5.	chicken with skin	6.03%
6.	white wine	4.78%
7.	apple juice	3.65%
8.	coffee	3.60%
9.	other fish	2.86%
10.	cooked cereal	2.51%

The estimated mean dietary exposure to total As in the U.S. is 50.6 µg/day for females and 58.5 µg/day for males (ATSDR, 2007), and typical consumption of inorganic As in is estimated between 8 and 14 µg/day (Yost et al., 1998). According to the U.S. Department of Agriculture's 1987-1988 Nationwide Food Consumption Survey, daily dietary intake of total As (Table 3.2) ranges from about 2 µg/day for infants to 92 µg/day for men aged 60-65 years (Tao and Bolger, 1999).

TABLE 3.2: Estimates of daily total and/or inorganic arsenic intake from food.

	Subgroup	µg/day	Reference
Dietary Total As	Children Age 6 Age 10 Boys,14-16 Girls, 14-16	23 20 13 15 21	(Tao and Bolger, 1999) Total Diet Study, 1991-97
	men 25-30 women 25-30 men 40-45 women 40-45 men 60-65 women 60-65 men, age 70 women, age 70	57 28 47 37 92 72 69 42	(Tao and Bolger, 1999) Total Diet Study, 1991-97
Dietary Total As	Males,25-30 1984-86 1986-91 1991-96	 58.1 38.6 56.6	(Gunderson, 1995b) (Gunderson, 1995a)
Dietary Total As	Adult Males Females	 45.7 39.7	(MacIntosh et al., 1996)
Dietary Total As		27.8	(Ryan et al., 2001)
Dietary Inorganic As	Adult	11.7-280	(Tao and Bolger, 1999)
Dietary Inorganic As	Adult	1-20	(Schoof et al., 1999)

IV. Metabolism and Biomarkers of Exposure

In some mammals, arsenic has a beneficial physiological role involving metabolism of methionine and the regulation of gene expression (Trumbo et al., 2001). There are major differences, however, in the absorption and metabolism of inorganic arsenic among mammals, and no other species appear to provide a good quantitative model for human arsenic metabolism (Vahter, 2002; Hughes, 2006) (ATSDR, 2007). Although a nutritional role for arsenic in humans has been postulated (Schoof et al., 1999), no studies have shown any indication of a beneficial role (ATSDR, 2007). A controversy regarding a dose-response relationship versus a threshold effect for arsenic toxicity and carcinogenicity in humans is ongoing. Proponents of a linear dose-response to As contend that there is no safe level of exposure (Smith, A. H. et al., 1992; Hopenhayn-Rich et al., 1993; Carlson-Lynch et al., 1994; Beck et al., 1995; Hopenhayn-Rich et al., 1996c). Opponents of the dose-response model contend that there is some threshold effect, below which most of the arsenic ingested is methylated and (presumably) detoxified (Carlson-Lynch et al., 1994).

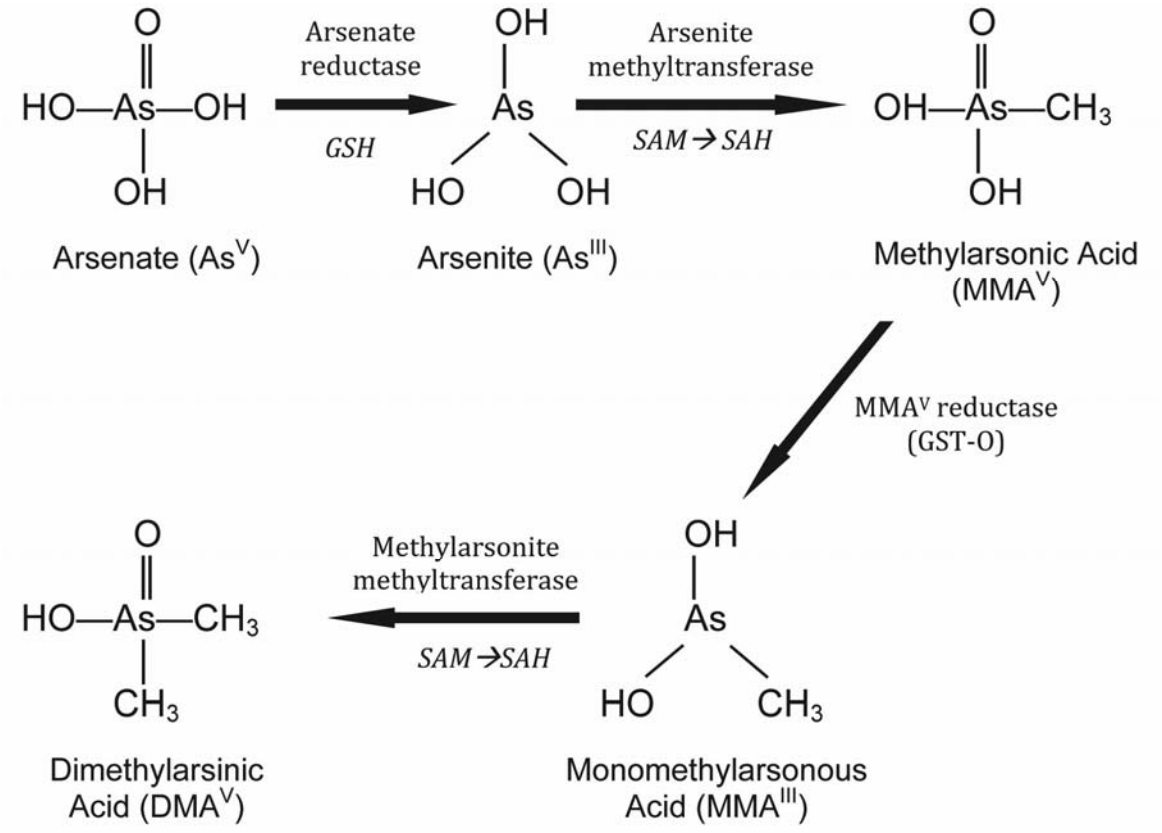
A. Human metabolism of arsenic

Natural organic arsenics, such as arsenobetaine (AsB), arsenocholine (AsC) and arsenosugars, occurring in seafood and seaweed, are efficiently absorbed and rapidly excreted in the urine (Suzuki et al., 2002). AsB and AsC appear to be excreted intact and are thought to have low toxicity. Dose-response toxicity of arsenosugars, however, is uncertain, and human ingestion of arsenosugars has been associated with increased urinary DMA (Suzuki et al., 2002).

In contrast, inorganic arsenic and metabolites are associated with substantial acute and chronic toxicity (Suzuki et al., 2002). Inorganic arsenic compounds are well-absorbed by humans through ingestion, with absorption values estimated at between 50 and 95% (ATSDR, 2007). Despite extensive study in animals and humans, metabolism of inorganic arsenic is not well understood. Two primary processes are involved (Figure 4.1), 1) reduction/oxidation reactions and 2) methylation reactions. The methylation of inorganic arsenic occurs in the liver and involves the 1-carbon metabolic pathway. Through this pathway, arsenate and arsenite are interconverted, through reduction reactions and methylation, to MMA^{+5}, which undergoes

reduction to MMA^{+3}, further methylation to DMA^{+5}, and possible further reduction to DMA^{+3} (this last step is uncertain in humans) (Steinmaus et al., 2005a).

Figure 4.1. Arsenic methylation pathway (Gamble et al., 2006; Lorenzana RM, 2009; Reichard and Puga, 2010).



GSH: glutathione; GSSG: glutathione disulfide; SAM: s-adenosyl methionine; SAH: s-adenosyl homocysteine; GST-O: glutathione-S transferase omega (=MMA^5 reductase)

Urinary As contains all of these forms in various proportions, but the trivalent methylated forms are short-lived and not readily measured. There are marked differences in toxicity of the metabolites, and the presence and proportions of the various urinary As species can be used as biomarkers of methylation efficiency and as potential health indicators (Steinmaus et al., 2005a) (Hopenhayn-Rich et al., 1993).

As compared with other animal species studied, humans typically excrete a greater proportion of ingested inorganic arsenic as MMA, possibly reflecting slower methylation of inorganic arsenic in humans than in rabbits, rats, and mice, and increased toxicological effects (Vahter, 2002; Hughes, 2006).

The sum of inorganic As and metabolites (As^{+3} + As^{+5} + MMA^{+5} + DMA^{+5}), referred to as arsenic sum of species, is used to approximate the total absorbed dose of inorganic As (Hopenhayn-Rich et al., 1993). The proportion of MMA to arsenic sum of species in the urine is a measure of the extent to which As is methylated only once prior to excretion. The ratio of MMA to inorganic arsenic (As^{+3} + As^{+5}) is considered the primary methylation ratio, and the ratio of DMA:MMA is the secondary methylation ratio.

Increased proportions of urinary MMA in areas with heavily As-contaminated drinking water are associated with greater toxicity and greater risk of arsenicosis (arsenic poisoning), skin and bladder cancers, and skin lesions (McCarty et al., 2007; Kile and Ronnenberg, 2008).

Although the metabolism of inorganic As facilitates excretion of As from the body, it also produces, as mentioned above, the trivalent methylated As compounds, MMA^{+3} and DMA^{+3}. These products are potentially even more cytotoxic, genotoxic, and more reactive than inorganic As (Cohen et al., 2006). It has been recently posited that methylation may not be entirely a detoxification process, as was previously thought (Hughes, 2006; Thomas, D. J. et al., 2007).

In cellular studies, arsenate replaces phosphate, thereby interfering with ATP synthesis and energy production, while arsenite interacts with thiol-containing amino acids, peptides, and proteins. Arsenite also binds to key sulfhydryl groups, causing enzyme inhibition (Ratnaike, 2003). Glutathione (GSH), a thiol peptide and antioxidant, appears to be involved in the reduction of As^{+5} to As^{+3}. S-adenosyl methionine (SAM) provides the methyl group for methylation (by arsenite methyltransferase and MMA

methyltransferase, respectively) to MMA and DMA (Cohen et al., 2006). Low levels of GSH are associated with higher arsenic toxicity, and diets low in sulfur amino acids (or low-protein diets in general) appear to suppress GSH levels and arsenic reduction (Peraza et al., 1998).

B. Genetic variation in arsenic metabolism

There are marked differences in individual susceptibility to the health effects of As. These differences may be due in part to a number of gene variants associated with arsenic metabolism and methylation efficiency (Abernathy et al., 1999; Yu et al., 2003; Meza et al., 2004; Lindberg et al., 2007; Meza et al., 2007; Schlawicke Engstrom et al., 2007). Glutathione S-transferase (GST) is part of a family of enzymes involved in metabolism and detoxification of arsenic and in cellular protection against reactive oxygen species (McCarty et al., 2007; Meza et al., 2007). Polymorphisms in the GST gene (GSTO1, GSTM1, GSTT1) may modify the risk of disease in arsenic-exposed individuals by modifying cellular levels of glutathione. Certain variants of the arsenite methyltransferase (AS3MT) gene are associated with lower levels of MMA^{+5} (greater methylation efficiency), and may be protective against arsenic-induced

disease (Meza et al., 2007; Schlawicke Engstrom et al., 2009; Gomez-Rubio et al., 2010). Variants of several other genes were associated with small effects on arsenic metabolic efficiency in specific populations and/or age groups, including genes affiliated with 1-carbon metabolism and production of SAM, 5-methyltetra-hydrofolate-homocysteine methyltransferase and choline dehydrogenase (Schlawicke Engstrom et al., 2009).

Epigenetic effects of inorganic arsenic exposure have also been hypothesized. Arsenic causes the depletion of SAM, the primary cellular methyl donor, which inhibits expression of DNA methyltransferase (Reichard et al., 2007). DNA hypermethylation in the promoter region of tumor suppressor genes in peripheral blood was observed in people chronically exposed to arsenic in drinking water (Chanda et al., 2006), revealing a mechanism for As carcinogenesis.

C. Nutrient pathways

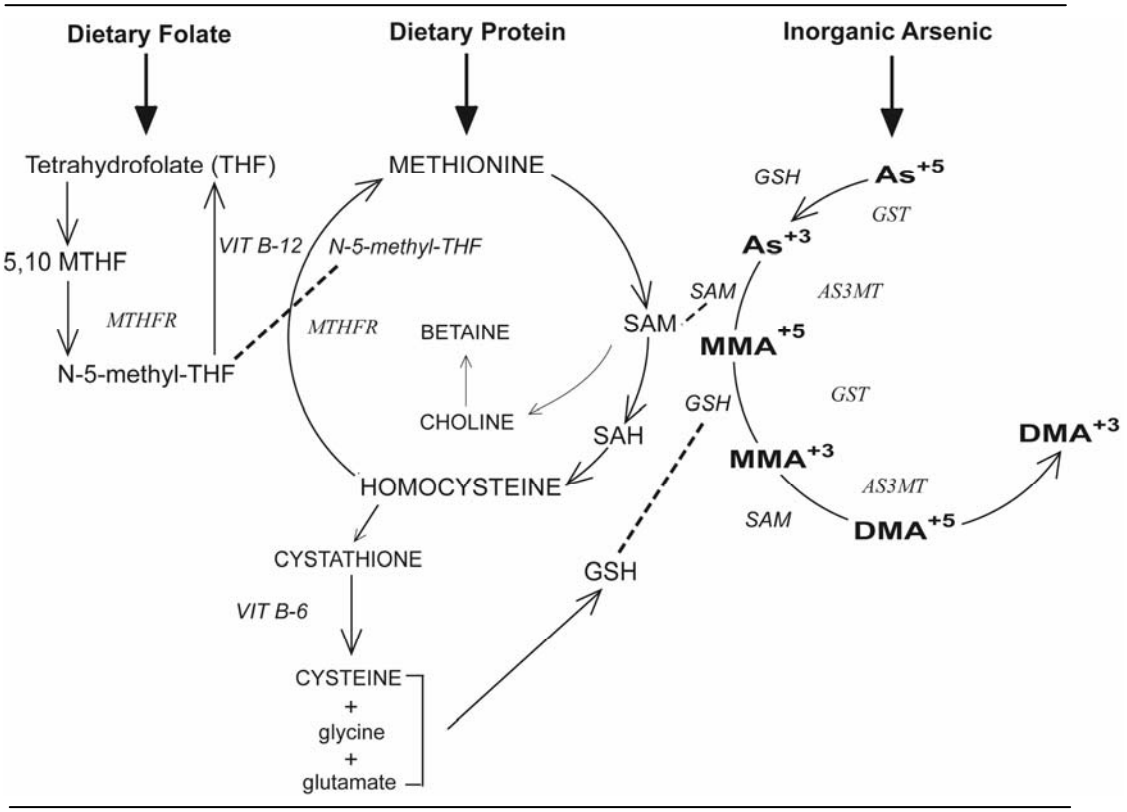
Nutrients, macro and micro, may interact with arsenic and either affect the toxicity or modify the body's response to arsenic by altering metabolism and transport (Kile and Ronnenberg, 2008). One-carbon metabolism converts inorganic arsenic to MMA and DMA and involves a folate-dependent

biochemical pathway that is responsible for the methylation not only of arsenic, but of DNA and hundreds of other molecules (Gamble and Liu, 2005; Kile and Ronnenberg, 2008) (see Figure 4.2). This pathway requires an adequate intake of folic acid, vitamin B_{12}, and choline for the re-methylation of homocysteine back to methionine (Vahter, 2007). Some evidence of interactions between dietary arsenic and dietary or blood levels of protein, folate, methionine, choline, cysteine, selenium, zinc, specific vitamins and other nutrient components has been observed (Schoof et al., 1999; Styblo and Thomas, 2001; Thomas, D.J. et al., 2001; Gamble et al., 2005; Steinmaus et al., 2005b; Heck et al., 2007).

Folate is a water-soluble B vitamin. Found in leafy green vegetables, citrus, and legumes, and added to breakfast cereals, breads and other products in the U.S., folate is the initial methyl donor in methionine biosynthesis. Methionine is activated by adenosine triphosphate (ATP) to generate S-adenosylmethionine (SAM), a process that also requires vitamin B_{12}, and SAM is the universal methyl donor in both arsenic metabolism and DNA methylation (Kile and Ronnenberg, 2008). Dietary methionine may modify DNA methylation, either by altering gene

expression in arsenic metabolizing enzymes, or by sparing folate, which is required for DNA synthesis and repair (Kile and Ronnenberg, 2008).

Figure 4.2. One-carbon metabolism, arsenic methylation, and relation to methionine and folate metabolism, after Vahter, 2007; Kile and Ronnenberg, 2008; Reichard and Puga, 2010.



GSH: glutathione, *GST: glutathione s-transferase,*
SAM: s-adenosyl methionine, *As3MT: arsenic methyl transferase,*
SAH: s-adenosyl homocysteine, THF: tetrahydrofolate, 5,10 MTHF: 5,10 methylene tetrahydrofolate, *MTHFR: methyl tetrahydrofolate reductase*

In a cross-sectional study of 1650 men and women in an area in Bangladesh with high water arsenic concentrations, the quantity of DMA excreted in urine was positively associated with plasma folate and negatively associated with homocysteine (Gamble et al., 2005). A separate study in Bangladesh in folate-deficient adults found that supplementation with folate reduced the proportion of MMA to total As in the urine (Gamble et al., 2006). In a highly exposed population in West Bengal, a slightly increased risk of arsenic-induced skin lesions was associated with the lowest quintile of intake of dietary animal protein (OR=1.94, 95% confidence interval, 1.05-3.59) and calcium (OR=1.89, 95% CI, 1.04-3.43) (Mitra et al., 2004). Basu et al. (2011) assessed methylation patterns and nutritional factors from the same population in West Bengal and found higher percent MMA (%MMA) in subjects with low serum folate and selenium. The interaction between arsenic metabolism and nutritional factors may be of greatest relevance in areas with poor nutrition.

V. Toxicity, Health Effects and Risk Assessment

Arsenic has been recognized as a human toxicant since ancient times. In an adult, a single exposure to 50,000-300,000 µg of As can be lethal, while in children, 3000 µg can cause death (Benedetti, 1996). Chronic exposure to > 10 µg of As per kilogram of body weight per day or > 100 µg As/L of water is associated with characteristic skin lesions and elevated risk of lung and bladder cancers, cardiovascular problems, and a number of other health problems (Taylor, 2004; ATSDR, 2007). Arsenic exerts its toxicity through inactivation of hundreds of enzymes involved in cellular energy pathways, DNA synthesis and DNA repair, and appears to be toxic to every organ system evaluated thus far (Ratnaike, 2003; ATSDR, 2007).

The Agency for Toxic Substances and Disease Registry (ATSDR) estimates an average daily adult exposure to As in the U.S. of approximately 50 µg/day from all sources (ATSDR, 2007). For a person weighing 150 pounds (68.0 kg), the average daily dose would be approximately 0.74 µg/kg/day.

Most studies on the toxicity and health effects of arsenic exposure have been conducted in geographic areas

with extremely high exposures, yet significant biological effects are observed in animal models and in cell cultures exposed to As at levels below the current EPA MCL of 10 ppb (Kozul et al., 2009). The estimated cancer-death risk associated with chronic exposure to inorganic As concentrations of 50 µg/L in water is 21/100,000 (Martinez et al., 2011). Mortality rates for cardiovascular, cerebrovascular, diabetes and kidney diseases appear to be elevated in populations exposed to median drinking water As concentrations of < 8 µg/L (Meliker et al., 2007), suggesting no lower threshold for toxicity. There is also substantial evidence of a high degree of variability in susceptibility to As-induced toxicity (Mushak and Crocetti, 1995).

A. Risk assessment

In 1975, EPA adopted the 50 ppb arsenic maximum contaminant level (MCL), based on the pre-existing (1942) standard of the Public Health Service. In a 1999 report, the National Research Council evaluated the epidemiological data linking arsenic exposure to public health effects, and recommended more stringent regulation based on findings of high cancer and non-cancer risks at the current standard

(NRC, 2001). Despite extensive criticism and controversy, EPA established a new standard of 10 ppb, based on estimates of excessive cancer risk at the previous federal standard of 50 ppb and new research suggesting a lifetime risk of bladder and lung cancer of 4-10 cases per 10,000 people with daily ingestion of 3 ppb arsenic. Public water systems in the U.S. were required to comply with the new arsenic standards by January 23, 2006. The NRC report noted that health risks associated with ingestion of arsenic from foods has not been studied and that risk assessments based only on ingestion of arsenic from drinking water are inadequate (NRC, 2001).

In 2010, the World Health Organization (WHO) established guidelines of a tolerable daily dietary intake level for arsenic of 2.1 µg/kg BW/day (ATSDR, 2007). This benchmark dose was estimated to be associated with a 0.5% increased incidence of lung cancer ($BMDL_{0.5}$). The median oral lethal dose (LD_{50}) of inorganic arsenic in humans is estimated to be 1-2 mg/kg, and the oral reference dose (RfD) considered safe for daily consumption is 0.3 µg/kg BW/day. The RfD is based on an estimated ingestion of 2 µg As/day from food and 4.5 L/day of water (Hughes, 2006).

Risk assessment for exposure to inorganic arsenic is based on extrapolation of risk from studies of highly-exposed populations to populations exposed to lower doses, and assume a linear dose, non-threshold model (NRC, 2001; Meharg and Raab, 2010). However, many uncertainties limit the accuracy of these risk estimates. Technologies for detection of low levels of arsenic metabolites are still being developed (Meharg and Raab, 2010), and the use of different technologies by different laboratories may lead to discrepancies in results (Kile et al., 2007a). Furthermore, animal models are not consistently relevant or suitable for extrapolating risks to humans, due to differences in the metabolism of As by other animal species (Vahter, 2002; Hughes, 2006; Meharg and Raab, 2010).

Present knowledge of arsenic toxicity and the mechanisms by which arsenic may induce health effects is not adequate for extrapolating low-dose effects. The shape of the dose-response curve and the application of different statistical models and/or models capable of accounting for factors that might affect susceptibility (i.e., age, gender, nutritional status, genetics, etc.) could markedly influence risk assessment (NRC, 1999). The shape of the dose-response curve is also likely to differ for different health effects

that may occur via multiple pathways, and via independent or interrelated mechanisms (NRC, 2001).

There are also uncertainties regarding the toxicity of endogenous (produced through internal metabolism of inorganic As) versus exogenous (external) sources of MMA and DMA raises the complex issue of whether arsenic methylation is necessarily a detoxification pathway (Cohen et al., 2006). Endogenous monomethylarsonic acid (MMA^{+5}) and dimethylarsinic acid (DMA^{+5}) are human metabolites of inorganic As and are readily absorbed (Cohen et al., 2006). In contrast, MMA and DMA from organic arsenical pesticides used for weed control do not appear to be readily absorbed and may be converted to carcinogenic inorganic forms (Cohen et al., 2006; Datta et al., 2006). Also, while organic and inorganic trivalent arsenates are generally considered more toxic than pentavalent forms, differences in relative potency are fairly small because of interconversion of arsenates and arsenites in the environment and in the body, and, in many cases, the precise speciation is unknown (Meharg and Hartley-Whitaker, 2002; ATSDR, 2007).

B. Health effects

Most of the epidemiological evidence for both carcinogenic and non-carcinogenic effects of exposure to As come from studies of populations exposed to levels greater than 300 µg/l in their drinking water, i.e., in Taiwan, Chile, India, etc. (Meliker et al., 2007). The American Council on Science and Health reported that there was distinct evidence that chronic exposure to inorganic arsenic at concentrations of several hundred micrograms per liter was causally related to: 1)cancers of the skin, bladder, lung, and possibly kidney, liver and prostate, 2)characteristic and distinctive cutaneous effects, i.e., diffuse or spotted hyperpigmentation and palmar-plantar hyperkeratosis, 3)peripheral vascular, cardiovascular and cerebrovascular disease, 4)diabetes, and 5)adverse reproductive outcomes (Brown and Ross, 2002). Additional systemic effects from both acute and chronic ingestion exposures have also been reported, including respiratory (Milton and Rahman, 2002; Parvez et al., 2010), gastrointestinal (Ratnaike, 2003), and hepatic (Hernandez-Zavala et al., 1998; Islam et al., 2011) effects in adults, and neurological (Guo et al., 2007; Yen et al., 2011) and intellectual deficits in children (Wasserman et al., 2004;

ATSDR, 2007; Mazumder, 2007). In a standardized mortality rate analysis in six Michigan counties, Meliker (2007) found significantly elevated mortality rates for cerebrovascular diseases, diabetes and kidney diseases associated with drinking water As levels between 10 and 100 µg/L.

Skin abnormalities associated with drinking arsenic-contaminated water have been recognized for decades and are a diagnostic sign of chronic arsenic toxicity (Tondel et al., 1999). These abnormalities include changes in skin pigmentation and keratosis that are generally observed after 5-10 years of chronic exposure, and prevalence is extremely high in communities exposed to arsenic-contaminated water in concentrations between 200 and 2000 µg/L. Exposure to drinking water with <50 µg/L of arsenic has been associated with relatively high rates of diagnostic skin abnormalities in several populations from different geographic localities (Rahman et al., 2009).

The health effects of low doses of arsenic in food and water are poorly understood due to lack of epidemiological data and the current state of scientific understanding of arsenic toxicity (Brown and Ross, 2002; Ahsan et al., 2006; Kile et al., 2007a). There is evidence, however, that

chronic exposure to > 8.1 µg/L of arsenic in drinking water is associated with skin lesions in Bangladesh (Ahsan et al., 2006), and that background exposure to levels below the current EPA limit are associated with increased risk of cancer (Tsuji et al., 2007).

Although inorganic arsenic is a known human carcinogen, it is a weak mutagen (Kitchin, 2001), and its carcinogenicity appears to result from either epigenetic changes in DNA methylation of oncogenes or tumor suppressor genes (Reichard and Puga, 2010), or a co-carcinogenic (co-exposure) effect (Hughes, 2002). Ingestion of arsenic has been associated with increased risk of skin, bladder and lung cancers, and possibly cancers of the liver, kidney, colon and prostate (Tchounwou et al., 2003). MMA^{+3} and DMA^{+3} in particular cause direct genotoxicity (ATSDR, 2007).

There is no consensus of opinion on potential pathways or specific mechanisms of action and no adequate animal models for health risks from ingestion of arsenic (Abernathy et al., 1999). Arsenite has been shown to cause inhibition of hundreds of enzymes, which may be an underlying cause of carcinogenic as well as non-carcinogenic effects (Abernathy et al., 1999), but other hypothesized mechanisms involve increased production of free radicals and epigenetic

effects on DNA and DNA repair mechanisms (Abernathy et al., 1999; Reichard and Puga, 2010).

Increased lipid peroxidation, superoxide production, hydroxyl radical formation, and oxidant-induced DNA damage have been observed at high doses in *in vivo* and *in vitro* studies of arsenic exposure, suggesting that oxidative stress may also be involved in arsenic toxicity, though the relevance to human exposure and physiology is, at best, suggestive (Yanez et al., 1991; Simeonova et al., 2000; Bongiovanni et al., 2007). There is supportive evidence that chronic, low-dose exposure can modify the expression of specific genes and proteins associated with oxidative stress and inflammation (Bongiovanni et al., 2007; Vahter, 2007; Lu et al., 2011).

In 1999, the National Research Council (NRC) subcommittee on Arsenic in Drinking Water concluded that additional epidemiological study was needed to clarify the relationship between arsenic and human health outcomes, especially at low doses (NRC, 1999; NRC, 2001).

C. Susceptible Populations/Risk Factors

There appear to be differences in the metabolism of arsenic between population groups and individuals (Vahter,

2002). Susceptibility to arsenic-induced dermatoses, pre-malignant skin lesions, gastrointestinal, and neurological effects may also vary by gender (Ahsan et al., 2006), age (Tsuji et al., 2004; Lindberg et al., 2008b), ethnic background, poverty, nutritional status, specific diets and genetic variants.

1. Children

Arsenic can readily pass through the placenta, and a few human studies have found evidence of elevated risk of impaired fetal growth and infant mortality with maternal arsenic exposure. Although infants appear to be partially protected by increased maternal methylation of As during pregnancy and lactation, and by low As excretion in breast milk (Vahter, 2009), researchers report a six-fold or greater increase in risk of stillbirth in West Bengal, India, at water As levels ≥200 µg/L, after adjustment for socioeconomic level, maternal age, body mass index, maternal and paternal education, and housing (von Ehrenstein et al., 2006).

There is relatively little data on health effects of arsenic exposure in children. In a review of the results from the US Food and Drug Administration (FDA) Total Diet

Study (TDS) from 1991-1996, 2-year-old children had a higher average dietary intake of total arsenic than any other age group, reaching 88% of the provisional tolerable daily intake (WHO, 1999; F.D.A., 2007). A market basket survey of rice in the U.S. showed that exposure to inorganic arsenic for infants exceeds the maximum intake of arsenic from drinking water (Williams et al., 2007). The acute health effects of arsenic exposure in children include gastrointestinal, neurological, and dermatological symptoms at exposure to ≥ 50 µg of As/kg/day (Tsuji et al., 2004). In Chile, subjects who were exposed *in utero* and in early childhood to very high levels of drinking water arsenic were at increased risk of mortality from lung cancer and bronchiectasis (Smith, A. H. et al., 2006).

The reported incidence of skin manifestations in arsenic-exposed children in different regions of the world varies between 1.9-37.1% (Mazumder, 2007). Chronic exposure in children has also been associated with developmental retardation (Mazumder, 2007), lower IQ scores (Bangladesh) (Wasserman et al., 2004) (Mazumder, 2007), and chronic lung disease, including pulmonary interstitial fibrosis (Chile) (Mazumder, 2007).

Metabolism of arsenic appears to be developmentally restricted, possibly due to genetic determinants. A large study in Bangladesh found a significant positive association between age and %MMA, suggesting decreased methylation efficiency with age (Lindberg et al., 2008a). In a population of 135 subjects in Sonora, Mexico, children between the ages of 7 and 11 were found to have a lower ratio of MMA^{+5} to total As in urine than adults, indicating higher methylation efficiency and lower risk of arsenic-induced disease. Furthermore, children with a specific variant of the arsenite methyltransferase (AS3MT) gene also had lower levels of MMA^{+5} (Meza et al., 2007).

2. Gender differences

Adult males have a higher rate of arsenic-induced skin lesions than adult females in communities with contaminated drinking water in Bangladesh (Tondel et al., 1999; Ahsan et al., 2006). The fraction of MMA in urine has been identified as a modifying risk factor, and a recent study of 1579 randomly selected individuals from a highly exposed area in rural Bangladesh found that the adjusted odds ratio of skin lesions in persons with urinary %MMA in the highest tertile is 2.8 (95% CI, 1.9-4.2) times that of persons with

urinary %MMA in the lowest tertile. The increased odds of skin lesions in males as compared to females are explained by decreased methylation efficiency in males (Lindberg et al., 2008a). However, in another analysis based on the same population, Lindberg et al. (2008a) found significant interaction between gender and age for %MMA, and the gender difference in methylation efficiency was observed only in females between 20-55 years of age, suggesting a possible role for sex hormones.

In the National Health and Nutrition Survey (NHANES) conducted in the U.S. in 2003-04, male/female differences in urinary As were observed, but racial/ethnic background may be an effect modifier. Mexican-American males had significantly higher creatinine-adjusted geometric mean total urinary As than Mexican-American females (P=0.005); however, non-Hispanic black males had lower total urinary As than non-Hispanic black females (P=0.038) (Caldwell et al., 2009).

3. Ethnic differences

There are marked geographic differences in exposure to arsenic and arsenic biomarkers, but ethnic differences within a geographic area have also been reported. As

mentioned above, Caldwell et al. (2009) report interaction between race/ethnicity and gender for total urinary arsenic levels in the 2003-04 NHANES study. Mexican-American males had significantly higher adjusted mean total urinary arsenic and urinary DMA than both non-Hispanic white males and non-Hispanic black males (Caldwell et al., 2009).

Some of the differences reported among ethnic groups may be attributable to both the geographic distribution of arsenic in soils and groundwater and to genetic variation. Other possible explanations of ethnic differences include cultural differences in diet, including foods consumed and/or how they are prepared, and differences in quantities of water consumed. Cultures with a fish-based diet or rice-based diet might have elevated levels of urinary total As, but speciated As might also be dependent on cultural and dietary differences. For example, Williams et al. (2007) modeled arsenic intake in the U.S. based on a market basket survey of rice and suggested that U.S. Hispanics and Asians might exceed the MCL from dietary intake of rice alone (Williams et al., 2007). Bangladeshis living in the United Kingdom have a 30-fold higher intake of rice than white Caucasians (Cascio et al., 2011).

4. Nutritional status

A number of studies, mostly in Bangladesh (Vahter, 2007), Taiwan (Chen, C. J. et al., 1988; Hsueh et al., 1995), and West Bengal (von Ehrenstein et al., 2006; Basu et al., 2011) report an association between poor nutrition (related to poverty) and arsenic-related health outcomes, possibly as a result of inadequate intake of specific nutrients involved in arsenic metabolism.

Other studies found an association between high body mass index (BMI) and increased arsenic methylation efficiency, especially in women (Lindberg et al., 2007; Schlawicke Engstrom et al., 2009; Gomez-Rubio et al., 2011). The mechanism by which high BMI may be associated with arsenic methylation efficiency is not understood (Schlawicke Engstrom et al., 2009; Gomez-Rubio et al., 2011). This association is not consistent across populations. In a study in rural Bangladesh, underweight individuals were no less efficient at methylating arsenic than the rest of the population (Lindberg et al., 2008b).

VI. Exposure and exposure modeling

In epidemiologic studies, the term exposure refers to any agent or factor that is associated with a health outcome, and can be an environmental factor, a biological or genetic marker, or an intervention (Last, 2001; ATSDR, 2007). In environmental toxicology, exposure is defined as contact between a chemical, physical or biological agent in an environmental medium (e.g., food, water, air, soil) and a target (e.g., a human or a specific organ). A more quantitative definition involves measurements of the concentration of the agent and/or the frequency or duration of contact (Zartarian et al., 1997). The methods used for measuring exposure status in an epidemiologic study include questionnaires, records, laboratory tests, physical measurements and other specific procedures. Information on frequency, duration, timing and extent of exposure increases the ability to quantify intake and the possibility of establishing a causal relationship between exposure and outcome (Kelsey, 1996).

A. Analytic methods

There are a variety of analytical methods used to determine the concentration of arsenic compounds in water, food, and urine. The costs, detection limits and other characteristics vary among methods. Inductively coupled plasma-mass spectrometry (ICP-MS)--the method currently approved by the U.S. EPA--can detect total arsenic down to 0.1 µg/L. Hydride generation-atomic absorption spectrometry (HG-AAS) is an earlier method used by EPA with a detection limit between 0.6-6 µg/L for total arsenic and arsenic speciation (IARC, 2004). Factors such as pH, iron content, temperature, methods of transport and sample preparation can affect the reliability of any of these methods.

Analysis of urinary arsenic species requires that the concentration of individual species not be changed by transport, handling and/or processing of the samples. As^{+5} undergoes rapid reduction to As^{+3} (Gong et al., 2002). The pentavalent arsenic species, MMA^{+5} and DMA^{+5}, are stable for up to approximately 4.5 months, but the trivalent arsenic species, MMA^{+3} and DMA^{+3}, thought to be key metabolic intermediates in human urine, are extremely unstable (Gong et al., 2002; Mandal et al., 2004). In urine samples stored

between +4°C and −20°C over a 5 month period, over 90% of MMA^{+3} was rapidly oxidized to MMA^{+5}, while DMA^{+3} was completely oxidized to DMA^{+5} within one day (Gong et al., 2002). Recently developed methods can preserve approximately 80% of the DMA^{+3} after 3 weeks of storage but only 10–24% after 4 months of storage (Jiang et al., 2003).

B. Modeling ingestion

Determining exposure to arsenic or other contaminants in water and food involves not just determining the concentration of the contaminant in the media, but also the volume of food and water ingested (O'Rourke et al., 1999a). The other critical component involves the time interval over which intake occurred. Short-term or instantaneous exposure versus long-term or chronic exposure, and average or median versus maximum exposure may be important in assessing exposure risks (Zartarian et al., 1997).

1. Arsenic in water

Various researchers have assessed the effects of water arsenic on urinary arsenic outcomes and arsenic-related health effects in different populations. While some

studies have carefully modeled exposure based on the concentration of As in drinking water sources along with individual water consumption volumes (Calderon et al., 1999; Lewis et al., 1999; Yoshida et al., 2004; Meliker et al., 2010a), others have estimated exposures on mean As concentration of a single source (Chen, Y. et al., 2011) (Marshall et al., 2007). The validity of a particular approach may depend on the extent of variation in arsenic concentrations from different sources, within a geographic area, and over time, as well as on the measurement instruments used and limits of detection (Yoshida et al., 2004; Meliker et al., 2010b).

2. Dietary exposure modeling

a. Types of dietary data

There are advantages and disadvantages to the various methods used to evaluate total dietary intake, and biases associated with each. Duplicate diet methods are assumed to be the "gold standard," especially for assessment of personal dietary contaminant intake, in that the method of collection is straightforward and the collected foods are portions of the same foods eaten, prepared in the same

facilities, and then analyzed for contaminants (Thomas, K.W. et al., 1997).

Food diary or food record methods generally involve completion of a detailed list of all of the foods consumed and the quantities consumed during the course of one or more days. Ideally, the subject records the foods at the time it is eaten (i.e., at each meal) to minimize recall error (Willett, 1990). Another instrument for assessing short-term dietary intake is the 24-hour recall, self-administered or administered by a trained interviewer, in which a subject is asked to recall everything eaten and drunk, including the quantities, over the previous 24 hours. This type of instrument requires good short-term memory, including accurate quantification of the amounts of each food consumed (Willett, 1990).

Food frequency questionnaires or diet histories are considered long-term instruments of assessment in that they are generally intended to represent "usual intake" over a longer and less precisely defined time period (Willett, 1990).

There are many sources of error associated with dietary recall and diet diary methods. These include under-reporting, either non-differential (i.e., measurement error

due to inadvertent or careless omissions and/or difficulties in estimating quantities eaten) or differential (i.e., dependent on the type of food eaten and psychological or sociological biases). On average, men and women under-report caloric intake by approximately 15-20% (Willett, 1990; Poslusna et al., 2009). Other concerns include representativeness of the day of record, but various methods have been developed—from collecting records on multiple days and multiple seasons to statistical modeling—that can reduce error (Dodd et al., 2006).

Physiologically implausible energy intake can result from either failure to record all items eaten and/or the amount eaten (under-recording), consuming less than the usual amount or less than that required to maintain body weight (under-eating), or a combination (McCrory et al., 2002). Over-reporting is less common, but results from overestimating the number of items and quantity consumed. Also, individuals on special diets, both weight loss and weight gain, may appear to be reporting implausible energy intake.

New approaches to reduce these errors are currently being explored. The National Cancer Institute is developing methods that utilize internet technology in which a

photograph of a "meal" is taken before and after it is consumed, and the food items and quantities are estimated by a computer software application (NCI, 2011). Similar approaches are under development by other groups and may help reduce reporting bias.

b. Food residue databases

The U.S. Food and Drug Administration (FDA) Total Diet Study (TDS) has been monitoring the US food supply for levels of nutritional elements and toxic chemical contaminants, including toxic elements, pesticide residues, and industrial chemicals, since the early 1960's (FDA, 2009). Currently, approximately 280 core foods representing the foods commonly consumed by the U.S. population are purchased at grocery stores and fast-food restaurants from four different parts of the country, four times annually, prepared "table-ready," and composited with two other samples from the region before being analyzed at the FDA's Kansas City District Laboratory in Lenexa, KS. The methods used have been relatively consistent over time, although the types and number of foods have changed gradually as food consumption patterns have changed in the U.S. The FDA assesses food consumption patterns based on U.S. Department

of Agriculture (USDA) surveys (the 1987-88 Nationwide Food Consumption Survey and the 1994-6, 1998 Continuing Survey of Food Intakes by Individuals) (FDA, 2009).

The FDA has used hydride generation atomic absorption spectrometry (HG-AAS) to measure total arsenic concentration in TDS since 1991. The limit of quantitation for arsenic in their analytical results from 1991-2005 was 0.12 mg/kg (120 µg/kg), and 88% of the FDA food samples from 1994-1997 had total arsenic that was below the limit of detection (LOD) of 0.04 mg/kg (40 µg/kg) (Egan et al., 2002). The mean concentrations reported were calculated by assigning all samples with non-detectable arsenic zeroes. Instead of assigning the LOD to non-detects, this approach was used to avoid overestimating exposure by assuming the presence of As in those foods (Egan et al., 2002). Median and maximum concentrations measured were also reported in the analytical results (FDA, 2009).

Schoof et al. (1999) used food consumption data from the U.S.D.A. Continuing Surveys of Food Intakes by Individuals (1992 through 1994), along with published surveys of total and inorganic As concentrations in food composites and food baskets. They selected 40 commodities that were predicted to account for 90% of dietary inorganic As intake in the

U.S. A modified market basket survey method was used to collect four food samples from supermarkets in Texas during October 1997 and the samples were left raw or prepared by microwaving (Schoof et al., 1999). Batelle Marine Sciences (Sequim, Washington) analyzed the samples for total As after NaOH digestion by ICP-MS and analyzed arsenic species using HCl digestion and hydride atomic absorption spectroscopy. Arsenic concentrations were averaged for all four samples, and in samples where arsenic was not detected or was below the detection limit, one-half the detection limit was assigned to the food. The LOD for total As was 3.6 ng/g (3.6 µg/kg) and 2 ng/g (2 µg/kg) for inorganic As (Schoof et al., 1999).

ICP-MS methods were used in the NHEXAS-AZ and ABS studies to analyze duplicate food and beverage samples. These had a limit of detection between 0.19-2.7 µg/kg.

c. Nutrient database

The Nutrition Coordinating Center (NCC) Food and Nutrient Database (University of Minnesota, Minneapolis, MN) is a comprehensive database of the nutrient composition of food. The database contains over 18,000 foods and enables users to select specific ingredients and preparation methods. It

currently includes over 160 nutrients and other food components. The Nutrition Data System for Research (NDS-R) software was also developed by the NCC and is designed for use with dietary recalls, dietary records, or diet histories. Data output is in Microsoft Excel for further analysis by the researcher (NDS-R, 2009).

The methods used by NCC for estimating nutrient content involve imputation procedures following specific protocols to reduce the number of missing nutrient values for less common foods or for uncommon preparation of foods in the database (Schakel et al., 1997). The database and software are updated continually.

C. Exposure modeling

A model is generally defined as a physical or conceptual representation or simplification of a complex reality. Statistical modeling involves fitting observational data to a mathematical function with parameters that can be adjusted so that the function closely resembles the empirical data (EPA, 2011). In contrast, a mechanistic model, such as a pharmacokinetic model, is characterized by compartmentalization of biological or physical processes

that attempt to represent the underlying mechanisms (Aarons, 2005).

Multiple regression analysis is a statistical method for studying the relationship between a single dependent variable and multiple independent variables. These types of models associate individual exposures and/or subject-specific risk factors to specific outcomes. They are used for: 1) prediction, i.e., to develop an equation for predicting the observed outcomes, and 2) causal analysis, i.e., to assess whether the independent variables are potential causes of the dependent variable, and to estimate the magnitude of the effect. Linear regression models are based on the assumption of a linear relationship between the dependent variable and the independent variables (Allison, 1999).

Physiologically-based pharmacokinetic (PBPK) modeling involves modeling the absorption and subsequent distribution, metabolism and excretion of pharmaceuticals, contaminants, toxicants, or other substances that leads to an understanding of the concentration of these substances or their biomarkers in the body (EPA, 2011). These complex models rely on a mechanistic understanding of the tissues and organ systems involved in absorption and metabolism and

attempt to account for individual variability in the dose-response relationship.

PBPK models are composed of a series of mathematical representations of physiological processes that simulate absorption, distribution, metabolism and excretion of chemicals that may enter the body through various pathways (i.e., ingestion, inhalation, dermal absorption, etc.), and estimate internal dose (referred to as a biologically effective dose) to target tissue(s). Utilizing differential equations that describe such processes as protein binding, membrane diffusion, metabolic clearance rates, etc. for exposures within each tissue compartment, the models describe changes in the quantity of chemical in the tissues over time (EPA, 2006). These models are often used in risk assessment. However, the validity of PBPK models is dependent on the extent and quality of the available data on which the models are based (Aarons, 2005).

The U.S. EPA has developed various Stochastic Human Exposure and Dose Simulation (SHEDS) models for dietary exposures (Xue et al., 2006; Zartarian et al., 2006). These probabilistic models simulate individual exposures to chemicals in food and drinking water. Modeling can accommodate specified time periods, such as 24 hours,

annual, or multiple years. Dietary consumption databases, rather than subject-specific data are used to develop these models (Xue et al., 2010).

PRESENT STUDY

Most prior research has emphasized the effect of drinking water arsenic on urinary arsenic excretion, and, indeed, existing federal regulations pertain to arsenic in drinking water, but not food. The overall goal of this research is to model the effects of dietary arsenic on biological markers that are indicators of arsenic-related health effects, so as to provide a basis from which public policy decisions can be made regarding the need to regulate arsenic in food.

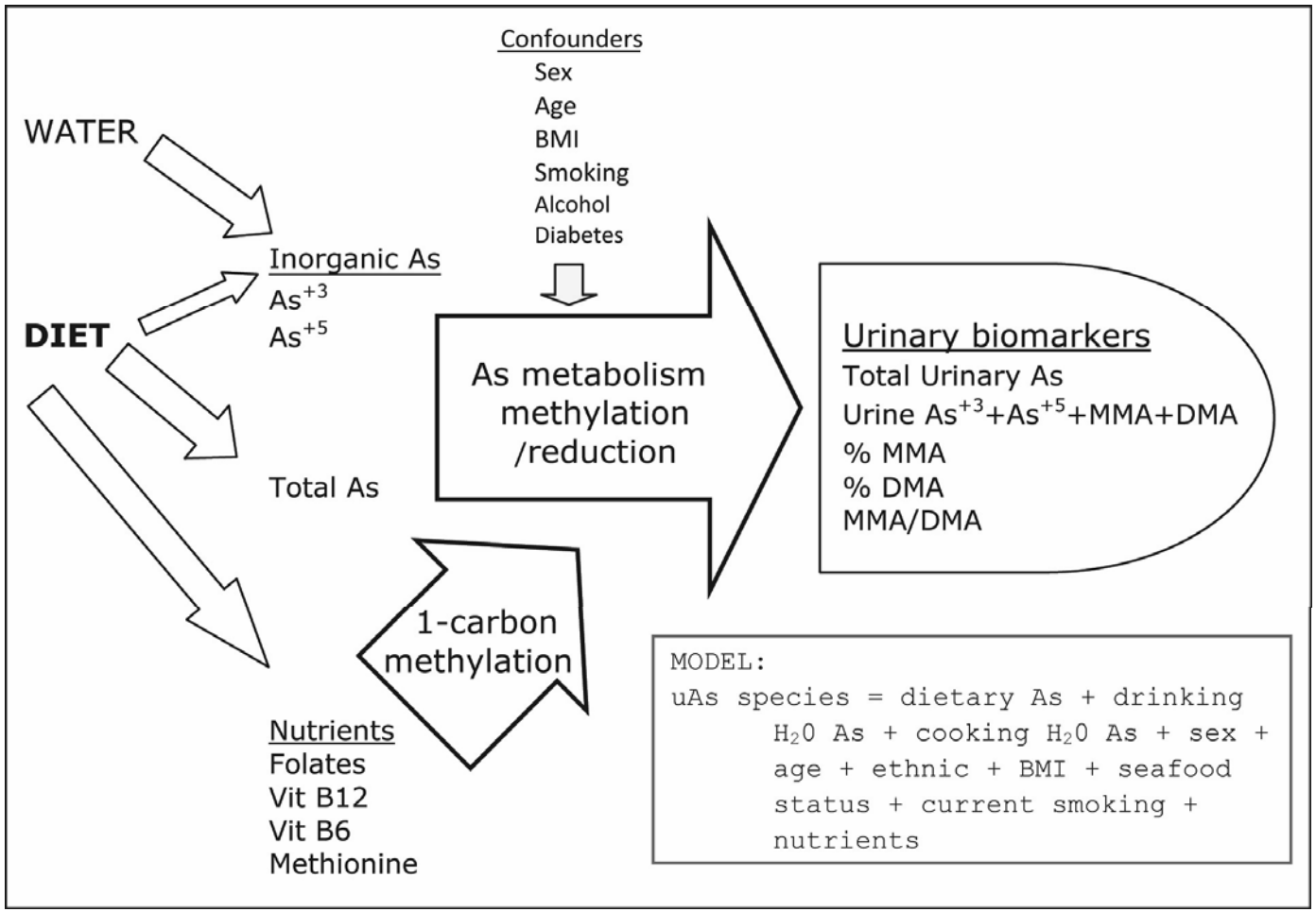
The aims of this study are to determine the relationship in local, regional and national study populations between dietary exposure to total and inorganic arsenic and urinary arsenic biomarkers. Three regional southwestern study populations that were well-characterized for exposure to arsenic are compared to each other and to a national population sample in this study. Arsenic in 24-hour duplicate diet samples was measured in the National Health Exposure Assessment Survey-Arizona (NHEXAS-AZ) and Arizona Border Survey. Arsenic exposure from food was modeled, based on 24-hour diet diaries, records and interviews used

in conjunction with published residue data in the Arizona sub-group of the Binational Arsenic Exposure Survey (BAsES) and in the 2003-2004 National Health and Nutrition Examination Survey (NHANES).

Each study collected first morning void urine samples from the population. In NHEXAS-AZ and ABS, urinary total As was measured; in BAsES and NHANES, total and speciated As (As^{+3}, As^{+5}, MMA, DMA, AsB, AsC) were analyzed. In the three regional populations, total As concentrations in multisource water samples were measured. Figure 7.1 illustrates a general schema of the causal pathway by which arsenic ingestion from diet and water influences the methylation of arsenic in the urine and may be modified by nutrient intake. Laboratory methods and the limits of detection for these methods are presented in Appendix A.

There is significant representation of Hispanic Americans (largely Mexican-American) in the regional databases, facilitating evaluation of the effect of Hispanic ethnicity in these models. Models stratified by household tap water As above versus below the MCL were assessed to evaluate the effectiveness of regulating water to reduce As dose.

Figure 7.1. Diagram of the causal pathway by which dietary and water arsenic intake and nutrient intake impact the methylation of arsenic in the urine.



VII. Overall Approach

Predictive models were developed that quantify the contribution of food and water to urinary concentrations of total and speciated arsenic. These arsenic exposure models were developed using local, regional, and national databases (Table 7.1 and Appendix B) that contained anthropometric, demographic and medical history information, urinary total and speciated arsenic measures, self-reported 24-hr diet data, and, in the local/regional studies, subject-specific information on water consumption, water sources, quantitation of total As in water used for drinking and water used for cooking.

All data were uploaded twice on the computer and compared for accuracy. Discrepancies were assessed and resolved when possible; questionable variables were flagged and documented. Evaluation of data included exploration of the study design, sample analysis and data quality indicators associated with specific values prior to data acceptance.

Table 7.1. Sources of data for modeling dietary contributions to arsenic exposure and methylation

URL/services	Media	Topic	Purpose
BAsES (Arizona)			
UA (Dr. Gandolfi) lab	water	arsenic	Inorganic arsenic
UA (Dr. Gandolfi) lab	urine	arsenic	Speciated arsenic
http://www.azcc.arizona.edu/research/shared-services/bmss	food	arsenic	Analysis of 24-hr dietary records
http://www.azcc.arizona.edu/research/shared-services/bmss	food	nutrients	Analysis of 24-hr dietary records
NHEXAS-AZ			
http://oaspub.epa.gov/eims/xmlreport.display?deid=23139&z_chk=69	water	arsenic	Inorganic Arsenic
http://oaspub.epa.gov/eims/xmlreport.display?deid=23142&z_chk=63814	food	arsenic	Total As, duplicate diet
http://oaspub.epa.gov/eims/xmlreport.display?deid=23126&z_chk=64834	food	arsenic	Diet diary
http://www.azcc.arizona.edu/research/shared-services/bmss			Total arsenic, diet diary
http://oaspub.epa.gov/heds/access_cmpl_dataset_frame?ds_id=23146&st_id=23159	urine	arsenic	Urinary total arsenic

Arizona Border Survey (ABS)			
Data files archived, Dr. M.K. O'Rourke[1]	water	arsenic	Inorganic arsenic
	food	arsenic	Total As, duplicate diet
	urine	arsenic	Urinary total arsenic
http://www.azcc.arizona.edu/research/shared-services/bmss	food	arsenic	Total arsenic, diet diaries
NHANES 2003-04			
http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/exam03_04.htm	diet	arsenic	Dietary interview, first day
http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/dr1tot_c.pdf	diet	nutrients	Total Nutrient Intakes, first day
http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06uas_c.pdf	urine	arsenic	Urinary speciated arsenic
ADDITIONAL DATABASES			
http://www.ncc.umn.edu/products/database.html	food	nutrients	Nutrition Data System For Research
http://www.fda.gov/downloads/Food/FoodSafety/FoodContaminantsAdulteration/TotalDietStudy/UCM243059.pdf	food	arsenic	Total Diet Study statistics on Element Results
Schoof et al., 1999	food	arsenic	Total and inorganic arsenic

[1] Arizona Border Survey (ABS) data have been provided to EPA for web listing (accessed original data archived by Dr. O'Rourke).

A. Study populations

National Human Exposure Assessment Survey (NHEXAS)-Arizona. NHEXAS was a multi-media, multi-pathway survey consisting of three separate studies, one of which was a population based probability design within the state of Arizona. Defining the human exposure distribution and identifying the 90th percentile of exposure to selected metals, pesticides and volatile organic compounds was the overarching goal of this study. General information was collected from subjects through questionnaires, time activity diaries, environmental (air, soil, house dust, food, beverage, tap and drinking water) and biological sampling. NHEXAS-AZ enrolled 1225 households and of these households, 179 were targeted for detailed, multi-analyte, multimedia sampling, which was conducted between 1995 and 1997. The study design, questionnaires employed, field and laboratory analytical techniques were described in a series of papers (Robertson et al., 1999; O'Rourke et al., 1999a; O'Rourke et al., 1999b).

Arizona Border Survey (ABS). The Arizona Border Survey enrolled 246 homes from 1997-1998 within 40 km of the US-Mexico border, with the purpose of evaluating differential

exposures in communities along the border. In general, the same recruitment, sampling strategy, questionnaires and sampling methodology were used as in NHEXAS-AZ, with detailed results obtained from 87 homes. Water, food and biomarker samples from both NHEXAS-AZ and ABS were analyzed by interagency laboratories (FDA, CDC, & EPA contract labs) with the goal of obtaining comparable measures.

Binational Arsenic Exposures Study (BAsES). The BAsES was designed as a cross-sectional survey of communities in northern Mexico and Arizona, representing a variety of groundwater arsenic concentrations, to evaluate the association between current arsenic exposure and metabolite profiles (Roberge, J. et al., 2012). Due to the absence of dietary arsenic residue data for foods purchased in Mexico, only data from Arizona residents were analyzed for this dissertation. In 2006, households from four communities in Arizona, two with high concentrations of As and two with low As concentrations in the municipal water supply, were randomly selected for recruitment. Adults over age 18 were eligible to participate. Participants provided urine, blood, buccal cell, and toenail samples, and completed questionnaires regarding environmental and occupational

exposures, drinking water consumption and 24-hour dietary records.

National Health and Nutrition Examination Survey (NHANES) 2003-2004. Conducted by the National Center for Health Statistics since 1971, NHANES is a cross-sectional, population-proportional national survey. The overall objectives of these surveys are to assess the health and nutritional status of adults and children in the United States. Since 1999, information has been collected from approximately 5,000 participants annually. NHANES uses a complex, stratified, four-stage probability cluster design, oversampling certain population subgroups to ensure representation (EPA, 2003).

The 2003-04 NHANES database included assessment of urinary arsenic and dietary nutrients and was used to validate the prediction models obtained from regional studies with a national population. In NHANES, subjects were interviewed at home regarding personal health history, demographics, and household characteristics. Detailed dietary interviews, physical exams, and sample collection were conducted in Mobile Examination Centers for persons over six years of age. Urinary speciated arsenic was

measured in a one-third subsample of persons ≥6 years of age, and specific sample weights for this subsample were used for analyzing the NHANES data (NHANES, 2007c). A total of 2420 subjects had both urinary As measures and completed 24-hr dietary interviews and were included in the analyses presented here.

Human Subjects Research Approval

All of the participants in the four study populations provided signed informed consent according to the requirements of either The University of Arizona Human Subjects Protection Program or the Centers for Disease Control/National Center for Health Statistics Ethics Review Board. Data from these approved studies were utilized in this dissertation research. This research involved secondary analysis of existing data, no recruitment was involved, and there were no personal identifiers in the data sets. The University of Arizona Human Subjects Protection Program granted this project an exempt status.

B. Dietary data collection methods

1. NHEXAS and ABS dietary data

In both NHEXAS AZ and ABS a 4-day diet diary, mapped to the USDA Total Diet Study, was completed. The diary consisted of a list of approximately 280 foods, organized by food type (i.e., dairy; breads, cereals, grains and pasta; etc.), and participants were instructed to fill in the foods and number of servings consumed at the end of each of the four days. The diary included the total number of servings and serving size for each food type, including tap and other drinking water.

Subjects were instructed to collect a duplicate sample of each food and beverage eaten over a 24-hour period. The sample was supposed to be an identical portion, prepared in the same way, including spices, sauces, etc. Solid food samples were collected in NHEXAS and ABS, but beverages (excluding drinking water) and food liquid at room temperature were only collected in NHEXAS, not in ABS. Subjects were also asked to complete a 24-hour diet record of all foods, beverages and drinking water consumed over the same 24-hour period. This record was compared in the laboratory with the food samples. Food type and weight

were recorded from the samples and the food was composited, shipped and analyzed for total arsenic content by an FDA laboratory.

2. BAsES dietary data

The BAsES study utilized 24-hour dietary recall questionnaires, administered by trained researches at a household visit. Participants were asked to describe all of the foods and beverages consumed over the previous 24 hours, preparation method, and the amount consumed. Portion-size models were used to facilitate estimation of quantity.

3. 2003-2004 NHANES dietary data

All participants in 2003-2004 NHANES were eligible to participate in the dietary interview survey. They were asked to recall the types and amounts of foods and beverages consumed during the 24-hour period prior to the interview. U.S. Department of Agriculture (USDA) developed a data collection instrument (the Automated Multiple Pass Method), which was used to collect detailed data on dietary intake (NHANES, 2007b; Moshfegh et al., 2008). Dietary interview data was coded using the USDA's Food and Nutrient Database for Dietary Studies, 2.0 (FNDDS 2.0) (USDA, 2006).

C. Analysis of dietary data

1. Adjusting for implausible energy intake

As part of the analysis of total nutrient intake (see section 4 below), total energy intake was assessed in all four study populations. The relation of BMI, age, sex, and ethnicity on reported energy intake was explored in each of the study populations to assess the need to adjust for physiologically implausible caloric intake (Huang et al., 2005). Standard equations were used (Mifflin et al., 1990), adjusting caloric intake only in those subjects who appeared to grossly over or underestimate intake.

To address missing data and extreme cases of over and under-reporting, prediction equations were used to calculate expected daily energy requirements (kcals/day), for adults (≥18 yrs) (Mifflin et al., 1990) and children (<18 yrs) (James et al., 1990; FAO/WHO/UNU, 2004). These equations are gender and age-specific and account for differences in body weight. For the purpose of this study, median basal metabolic rate was assumed for children by age and sex category; a sedentary lifestyle was assumed for adults. Based on the calculation of expected energy intake, a correction factor was then computed to adjust

dietary exposures for extreme under and over-reporting (McCrory et al., 2002). For subjects who reported consuming <75% of the kcals/day required to maintain body weight, the correction factor was equal to the expected kcals divided by the reported kcals. For subjects who reported consuming >150% of expected kcals, the correction factor was calculated as (1.25 x expected kcals)/reported kcals, allowing for the possibility that these individuals over-ate on the day of sampling. The correction factor was then used as a multiplier for calculating dietary exposure to arsenic and nutrients. Appendix C shows the detailed methods used to adjust for implausible energy intake, and Appendix D assesses and compares reported energy intake in the four study populations.

2. Dietary arsenic modeling

The methods used for modeling dietary arsenic involved: 1) analysis of 24-hour diet records, accounting for caloric misreporting, and 2) estimation of mean and maximum total arsenic ingested based on the Total Diet Study (TDS) Statistics on Element Results (Revision 4.1), and total and inorganic arsenic based on Schoof's "market basket survey" of total and inorganic As in food (Schoof et al., 1999).

Three different sources of residue data were used to estimate dietary intake of arsenic: 1) the Total Diet Study, 2) a published market basket survey of total and inorganic As in foods by Schoof and colleagues (1999), and 3) 24-hour duplicate food samples collected in NHEXAS-AZ and ABS. These sources are described below.

Evaluation of As content of diet diary, dietary recall, and dietary interview data for all of the study populations was conducted by the Diet and Behavioral Assessment Center at the Arizona Cancer Center according to the protocols in Appendix E and F.

The duplicate food samples collected in NHEXAS-AZ and ABS were sent on ice to the FDA, where they were homogenized and aliquoted prior to analysis. Total As concentrations of the composited samples were assayed using ICP-MS with a limit of detection between 0.19 and 2.7 µg/kg in food and beverage samples (O'Rourke et al., 1999a). Separate analysis of food and beverage samples were conducted in NHEXAS-AZ, but only solid food samples were analyzed in ABS.

3. Nutrient analysis

The Diet and Behavioral Assessment Center at the Arizona Cancer Center used the Nutrition Data System for Research (NDS-R) software, Version 2005 to analyze total caloric intake and dietary nutrient intake in NHEXAS, ABS, and BAsES. Dietary interviews and analysis of nutrient intake in NHANES were conducted by the USDA in partnership with the US Department of Health and Human Services. The nutrient amounts reported are the amounts in foods and beverages and do not include dietary supplements (NHANES, 2007a).

The nutrients used in modeling in this study were dietary folates, Vitamin B-12, Vitamin B-6, protein, and methionine. As discussed above, nutrient intake was adjusted for caloric under and over-reporting.

D. Water arsenic modeling

Samples of all water sources that subjects specified as used for drinking and/or cooking were collected in NHEXAS, ABS, and BAsES and analyzed for total As. No water samples were collected or analyzed for arsenic in the NHANES population.

NHEXAS and ABS drinking and cooking water samples were analyzed by EPA contract laboratories using ICP-MS, with a limit of detection of 0.20 µg/L (O'Rourke et al., 1999b). The average number of glasses of drinking water consumed per day was recorded in time-activity questionnaires, converted to liters/day, and multiplied by the concentration of As in the drinking water sample to estimate daily As exposure from drinking water in µg/L/day.

The quantity of water used in cooking was estimated for specific food items that were cooked at home and required water for preparation (for example, hot cereals, rice, pasta, dried beans, powdered drinks, soups made from concentrate, etc.). The number of grams of water added in preparation was determined from the NDSR output file for individual foods, which included a column for the number of grams of water per food code. (Cooking water As was not modeled for foods not cooked at home.) The percent of water per food item was calculated and used to compute the intake of grams of cooking water added in preparation, based on the total number of grams of that food consumed. Then, the total number of grams of water used for cooking was summed for each subject, and then multiplied by the As

concentration in the specific water source(s) used in food preparation.

BAsES water samples were analyzed by the Hazard Identification Core at the University of Arizona, supported by the Superfund Basic Research Program Grant from the National Institute of Environmental Health Sciences. Details of the methodology are presented elsewhere (Roberge et al., 2012, submitted manuscript), but, briefly, ICP-MS analysis was used, with a limit of detection of 0.10 µg/L for total As. All water sources used for drinking and cooking and the frequency of use of each source was reported. Subjects reported the quantity of drinking water consumed in the 24-hour dietary recall interviews. Arsenic exposure from drinking and cooking water in BAsES was estimated according to the methods shown in Appendix G, which were used to convert the frequency of use by source and As concentration by source to a weighted concentration. The weighted concentration was then multiplied by the quantity of drinking water consumed per day. A weighted mean As concentration based on frequency of use of multiple water sources was used to separately estimate total drinking and cooking water As exposure per day (µg/L/day). Missing water As concentration values were replaced with

the population mean concentration for water used for drinking and for water used for cooking. Missing values for volume of drinking water consumed were also replaced with the population mean value.

E. Urinary arsenic

In the BAsES, NHEXAS and ABS studies, first-morning void urine samples were collected by the participants and kept refrigerated until interviewers collected the samples. Similar processing protocols were used in all three studies. NHEXAS and ABS samples were analyzed for total arsenic by FDA using ICP-MS, which had a minimum detection limit of 4.1 µg/L (O'Rourke et al., 1999a; O'Rourke et al., 1999b). BAsES samples were analyzed for total arsenic and arsenic species (including As^{+3}, As^{+5}, MMA^{5}, DMA^{5}, and AsB) by Hazard Identification Core at the University of Arizona using ICP-MS. The detection limits were 0.12 µg/L for As^{+3}, MMA^{5} and DMA^{5}, and 0.21 for As^{+5}.

In NHANES 2003-2004, urinary arsenic was measured in a random subsample of one-third of survey participants aged six years and older. Analytic methods are described in detail in the urinary arsenic documentation (NHANES,

2007c). Briefly, both total and speciated arsenics were determined using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS). Detection limits were 1.0 µg/L for As^5, 1.2 for As^3, 1.7 for DMA^5, and 0.9 for MMA^5 (Caldwell et al., 2009).

Urinary As measures were adjusted for urine specific gravity in BAsES and both creatinine-adjusted and unadjusted urinary As measures were analyzed in NHANES (see Appendix H).

F. Statistical Analysis

Imported data from each study were tabulated and summarized. Subjects missing data on urinary or dietary As were excluded from analyses. Missing values for water As were estimated based on population means stratified by sex and/or age category. The distribution of variables related to As and micronutrient concentrations were assessed for normality and percentage of samples below the level of detection. If the assumption of normality was violated, the variable was either transformed, or divided into tertiles or other percentiles, depending on the distribution.

Standard statistical tests, including simple regression or simple mixed model regression, chi-squared, t-tests, analysis of variance (ANOVA), Wilcoxan rank-sum, and Kruskal-Wallis tests were used to assess the crude relationship between variables, differences by gender and ethnicity, and to help determine which variables to include in the multivariable models. A two-sided significance level of 0.05 was set *a priori* for all tests. Stata version 11.2 (StataCorp, 2011) was used for data analysis, and a two-sided significance level of 0.05 was set *a priori* for the regression models.

1. Outcome measures

Four urinary As biomarkers were explored as outcome variables in the regression models: urinary total arsenic, sum of species arsenic, percent MMA (% MMA), and the ratio of DMA to MMA (DMA:MMA). The formulas used for calculating these are shown in Table 7.2.

2. Covariates

Covariates in the models included drinking and cooking water arsenic exposure, ethnicity, age, sex, body mass

index (body weight in kg/height in m^2), cigarette smoking status (current vs. not current).

Table 7.2. Equations used to calculate urinary arsenic biomarkers.

Sum of species arsenic = As3 + As5 + MMA + DMA
% MMA = (MMA/(As3 + As5 + MMA + DMA))
MMA/DMA = MMA/DMA

3. Modeling strategy

Table 7.3 shows the various multivariable models that were tested to address the different specific aims. The effects of ethnicity on urinary biomarkers were evaluated in all study populations. Effect modification by household water As concentration, above vs. below 10 ppb and 5 ppb, was tested and models with and without interaction terms were compared using likelihood ratio tests ($P<0.10$ for interaction). The likelihood ratio test was also used to test for confounding in nested models, and Akaike's Information Criterion was used to compare non-nested models. R-squared values were calculated to determine the proportion of variance in the dependent variable accounted

for by the independent variables, and adjusted r-squared values to assess model fit.

Because of high organic arsenic levels in seafood, sensitivity analyses were run in which subjects who reported eating fish or other seafood in the previous 24 hours were excluded from the analyses in order to assess whether consumption of fish and/or shellfish altered the relationship between dietary As intake and urinary As excretion.

Additional methods used to test specific hypotheses are presented as study-specific methods under each specific aim in Chapter VIII.

Table 7.3: Multivariable models tested

AIM 1: NHEXAS-AZ and AZ Border Survey

Urinary Total As = [calorie-adjusted(g/day FOOD * dietary^2 As)]
+ (L/day drinking * total As) + (L/day cooking H_20 * total As)
+ sex + age + ethnic + BMI + current smoking status

STRATIFIED by tap water As concentration
STRATIFIED by ethnicity
RESTRICTED analysis (subjects who did not consume seafood)

Table 7.3 (continued)

AIM 2: BAsES

a) Effect of dietary As on urinary As methylation

Urinary As species[1] = [calorie-adjusted(g/day FOOD * dietary[3] As)] + (L/day drinking * total As) + (L/day cooking H_20 * total As) + sex + age + ethnic + BMI + current smoking status

STRATIFIED by tap water As concentration
STRATIFIED by ethnicity
RESTRICTED analysis (subjects who did not consume seafood)

b) Effect of dietary nutrient intake on urinary As methylation

Urinary As species[1] = [calorie-adjusted(g/day FOOD * dietary[3] As)] + (L/day drinking * total As) + (L/day cooking H_20 * total As) + sex + age + ethnic + BMI + current smoking status + [calorie-adjusted (μg/day MICRONUTRIENTS[4])]

AIM 3: NHANES 2003-2004

a) Effect of dietary As on urinary As methylation

Urinary As species[1] = [calorie-adjusted(g/day FOOD * dietary[3] As)] + sex + age + ethnic + BMI + current smoking status

STRATIFIED by ethnicity
RESTRICTED analysis (subjects who did not consume seafood)

b) Effect of dietary nutrient intake on urinary As methylation

Urinary As species[1] = [calorie-adjusted(g/day FOOD * dietary[3] As)] + sex + age + ethnic + BMI + current smoking status + [calorie-adjusted(μg/day MICRONUTRIENTS[4])]

[1]Urinary As sum of species, %MMA, DMA/MMA; [2]Dietary As predictors: duplicate diet, TDS mean total As, TDS maximum total As, Schoof total As and Schoof inorganic As; [3]Dietary As predictors: Schoof total As and Schoof inorganic As; [4]Micronutrients: Folate, methionine, Vitamin B-12, Vitamin B-6, protein

VIII. Study Results and Discussions

A. Specific Aim 1. *To compare measured versus modeled estimates of total dietary arsenic as predictors of total urinary arsenic in the NHEXAS-AZ and Arizona Border Study populations. Dietary arsenic intake was measured from 24-hour duplicate diet samples and modeled based on 24-hour diaries and published arsenic residue data from the U.S. FDA Total Diet Study (TDS) Statistics on Element Results (1991-2003)(F.D.A., 2007) and on a limited market basket survey of food commodities assumed to contribute 90% of dietary inorganic As (Schoof et al., 1999).*

The objectives of this study were to: 1) assess the validity of indirect methods of modeling 24-hr dietary As exposure by comparing modeled intake to measured intake, and 2) evaluate modeled versus measured dietary As intake as predictors of urinary total As excretion.

1. Rationale

There are difficulties inherent in modeling ingestion exposure, including extensive variability in As content of foods due to heterogeneity of source and differences in

preparation (Meharg and Raab, 2010; Xue et al., 2010). Current knowledge of dietary As exposure is limited by the difficulties of obtaining complete and accurate dietary intake information and local, regional, and person-specific sources that might contribute to differences in exposure (MacIntosh et al., 1996). Collecting and measuring duplicate diet samples is expensive and time consuming, especially in population studies. Hence, alternative methods of estimating dietary exposure need to be evaluated.

Several prior studies have compared different methods of modeling dietary exposure to As and have yielded disparate estimates of exposure. Dietary Exposure Potential Models (DEPM), based on diet records and As residue databases, were applied to NHEXAS data and compared to duplicate diet residue results, with only modest concordance (Georgopoulos et al., 2008; Xue et al., 2010). These studies do not adequately address demographic, regional, or person-specific differences in consumption patterns, nor do they address subject-specific use of local water sources for drinking and food preparation or implausible energy intake (Moschandreas et al., 2002). The current study presents a novel comparison of direct statistical modeling of urinary

arsenic excretion based on published residue databases.

2. Study-Specific Methods

For this study aim, the National Human Exposure Assessment Survey Arizona (NHEXAS-AZ) and the Arizona Border Survey (ABS) were initially analyzed separately and then later merged. Dietary total As was measured in 24-hour duplicate diet samples and modeled from the diet diaries. Subjects completed the diet diaries over the same time period that duplicate food samples were collected, and the diaries were then used to model ingestion of As from food. Arsenic content of the foods consumed were matched to contaminant residue data from two sources: 1) US FDA Total Diet Study (TDS) market baskets (1991-2005), mean and maximum total As concentrations (F.D.A., 2007), and 2) mean total and inorganic As from the market basket survey conducted by Schoof and colleagues (Schoof et al., 1999). Measured and modeled dietary As were adjusted for implausible energy reporting.

The limit of detection (LOD) of urinary total arsenic in this study was 4.1 µg/L and a large percentage of subjects had values below the LOD (36% in NHEXAS and 57% in ABS).

This necessitated a two-stage analytical approach, in which urinary As was initially evaluated as a dichotomous variable (above versus below the LOD), and then those subjects with urinary As values above the LOD were modeled separately. Dietary, drinking water, cooking water and urinary As values above the LOD were log-transformed for analysis. Covariates in the adjusted models included sex, Hispanic ethnicity (yes/no), and current smoking status (yes/no). Arsenic intake from water used in food preparation was not included as a separate covariate in the duplicate diet models, since duplicate food samples were "table-ready." Intraclass correlations between measured and modeled dietary As intake were estimated using maximum likelihood for variance components models, and Fisher's z-test was used to compare the equality of the intraclass correlation coefficients.

For NHEXAS and ABS, two-stage regression analyses were used to evaluate the relation between urinary total As and each dietary As exposure variable. First, simple logistic regression was used to model urinary As above versus below the LOD. Next, linear regression models restricted to subjects with urinary As above the LOD were run. Bootstrap sampling techniques were applied in the restricted analyses

to obtain conservative estimates of standard errors of the regression coefficients. Bootstrap sampling involved 500 simulations with replacement from the original dataset (including both observations above and below the LOD).

Two-stage mixed models for the combined population incorporated study population as a random effect, generalized linear mixed models in the stage 1 regression, and linear mixed models in stage 2. Likelihood ratio tests were used to compare models with and without additional covariates. To assess potential interaction, these same models were run in analyses stratified by: 1) tap water As concentration, above and below 10 ppb and 5 ppb, and 2) Hispanic vs. non-Hispanic ethnicity. Sensitivity analysis was run to assess whether consumption of seafood (fish and/or shellfish) modified the relationship between dietary and urinary As. A two-sided significance level of 0.05 was set *a priori* for the regression models.

3. Results

Population characteristics and exposures

Characteristics of each study population and of the combined population are shown in Table 8.1. Participants with urinary total As and duplicate diet data numbered 166 in the NHEXAS-AZ and 86 in ABS. NHEXAS was a statewide survey and had significantly fewer Hispanics (28% vs. 79%) and a smaller proportion of females (60% vs. 72%) than ABS. Although age range was similar in both studies, NHEXAS had a greater proportion of children ≤ 18 yrs (20% vs. 2%) and mean age in NHEXAS was 41.6±20.5 vs. 48.4±15.3 yrs in ABS.

In both studies, the concentration of As in water used for drinking and cooking was generally below 5 µg/L. The amount of drinking water consumed was significantly lower in ABS than in NHEXAS (1.07 vs. 1.77 L/day, $P<0.001$), as was the concentration of As in the drinking water (0.68 vs. 1.23 µg/L, $P=0.003$). The As content of water used for food preparation was similar in both studies (4.50 vs. 4.17 µg/L, ABS and NHEXAS, respectively). In ABS, 57% of subjects had urinary As below the LOD, as compared with 36% in NHEXAS ($P=0.002$). The maximum concentration of As in urine was 340 µg/L in ABS and 430 µg/L in NHEXAS.

Table 8.1. Population characteristics in NHEXAS-AZ, ABS and in both studies combined.

	NHEXAS-AZ	ABS	COMBINED POPULATION
N of subjects	166	86	252
N (%) female	99 (59.6)	62 (72.1)	169 (64.3)
N (%) Hispanic	47 (28.3)	68 (79.1)	141 (53.8)
N (%) current smokers	32 (20.3)	11 (12.8)	47 (18.5)
N (%) consumed seafood	20 (12.0)	14 (16.3)	34 (12.93)
N (%) urinary As < LOD[1]	60 (36.1)	49 (57.0)	109 (43.3)
Age, years (mean ± SD)	41.59±20.47	48.41±15.33	43.92±19.12
(minimum-maximum)	6-83	10-79	6-83

Table 8.1 (continued)

	NHEXAS-AZ	ABS	COMBINED POPULATION
Drinking water, L/day			
(geometric mean, 95% CI)	1.77 (1.60-1.95)	1.07 (0.96-1.19)	1.48 (1.37-1.61)
Cooking water, L/day			
(geometric mean 95% CI)	0.56 (0.48-0.64)	0.20 (0.17-0.25)	0.40 (0.35-0.46)
Urinary As, µg/L			
(geometric mean, 95% CI)	9.05 (7.53-10.86)	6.50 (4.87-8.67)	8.08 (6.92-9.44)
N (%) ≤ LOD[1]	49 (57.0)	60 (36.1)	109(43.2)

[1] LOD = limit of detection (4.1 µg/L urine)

In NHEXAS, 75% of subjects consumed more than 1.5 times the expected number of kilocalories, and average caloric intake was almost twice that in ABS (see Appendix D). No duplicate beverage samples were collected in ABS, but beverage consumption was reported in the diaries and represented in the modeled dietary exposure estimates. Despite this discrepancy in either kilocalorie intake or reporting between studies and the absence of duplicate beverage samples, ABS had significantly higher measured and modeled dietary As exposure estimates than NHEXAS.

Arsenic exposure estimates for the combined population and stratified by study and Hispanic ethnicity are presented in Table 8.2. Modeled estimates of mean 24-hr dietary As exposure varied by method of determination, but TDS maximum (TDS max) total As estimates were the closest approximation to measured dietary total As in each population and in the merged population. In the combined population, mean exposure based on duplicate diet was 43.3 µg/day and 51.9 µg/day based on the TDS max. Applying Schoof mean total As data to the diet diaries (Schoof total) yielded the highest estimate of intake—approximately 64 µg As/day in the combined population. In contrast, TDS means estimated dietary As intake at only 8.2 µg/day.

Dietary As exposure was higher in ABS than in NHEXAS. Overall, Hispanics in the combined population had significantly elevated exposure to dietary total As and lower exposure from drinking water than non-Hispanics (all P <0.01).

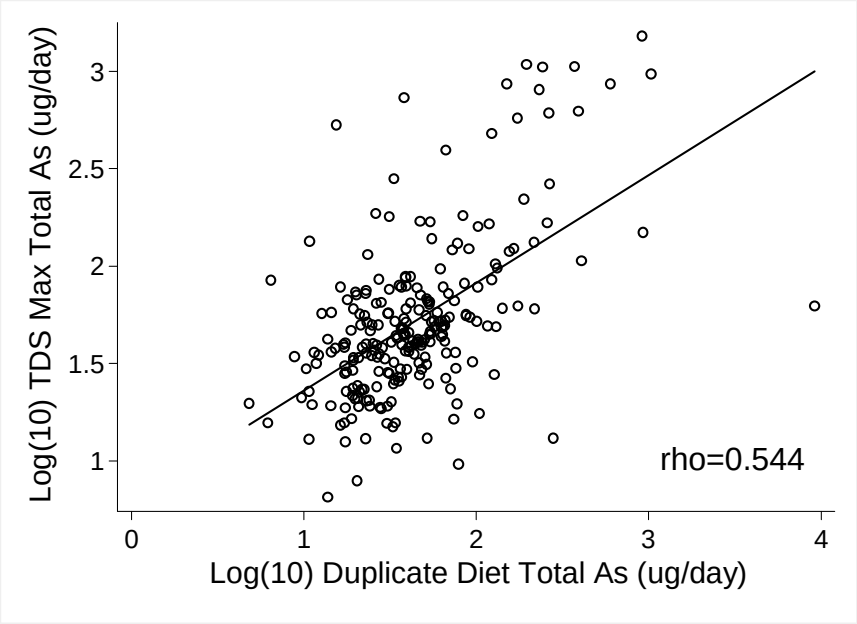
Despite differences in the mean estimates, TDS max and Schoof total dietary As were significantly correlated with each other and the intraclass correlation coefficient (rho) was 0.780 (P<0.001). The correlation between TDS max and duplicate diet As was 0.544, and between Schoof total and duplicate diet was 0.451 (both P<0.001) (Figure 8.1a and b). TDS mean dietary As was not significantly correlated with duplicate diet As (not shown).

Table 8.2. Dietary and water arsenic exposure in the combined population and stratified by study population and Hispanic ethnicity (geometric means, medians and range).

	Dup Diet Total As µg/day	TDS Total As (mean) µg/day	TDS Total As (maximum) µg/day	Schoof Total As µg/day	Schoof Inorganic As µg/day	Drinking Water Total As µg/day	Cooking Water Total As µg/day
COMBINED POPULATION							
Geometric mean	43.29	8.23	51.92	63.80	8.35	1.46	1.73
Median	38.15	7.97	45.07	58.31	7.27	1.51	1.40
Range	4.85-9190	0.23-438	6.49-1510	14.29-1632	2.16-65	0-140	0-200
NHEXAS-AZ							
Geometric mean	33.92	8.69	44.65	54.83	7.17	2.09	2.46
Median	30.69	9.34	41.88	54.58	6.76	2.98	2.42
Range	4.85-9190	0.31-375	6.49-730	14.29-225	2.16-46	0-140	0-200
ABS							
Geometric mean	67.57	7.42	69.47	85.47	11.21	0.73	0.84
Median	54.24	5.49	48.51	76.77	9.61	0.65	0.87
Range	11.79-1030	0.23-438	11.55-1510	15.23-1632	3.01-65	0.02-33.5	0-6.57

Table 8.2 (continued)

	Dup Diet Total As µg/day	TDS Total As (mean) µg/day	TDS Total As (maximum) µg/day	Schoof Total As µg/day	Schoof Inorganic As µg/day	Drinking Water Total As µg/day	Cooking Water Total As µg/day
Hispanics							
Geometric mean	52.90	9.18	61.15	76.79	9.76	0.90	1.46
Median	50.13	8.54	48.63	68.65	8.92	0.69	1.29
Range	4.85-1030	0.23-414	9.61-1510	15.23-1632	2.19-65	0-45.99	0-39.62
Non-Hispanics							
Geometric mean	37.16	7.57	45.61	54.92	7.36	2.15	2.04
Median	32.40	7.86	41.00	52.06	6.76	2.69	1.97
Range	6.49-9190	0.31-438	6.49-1078	14.29-868	2.16-55	0-140	0-200



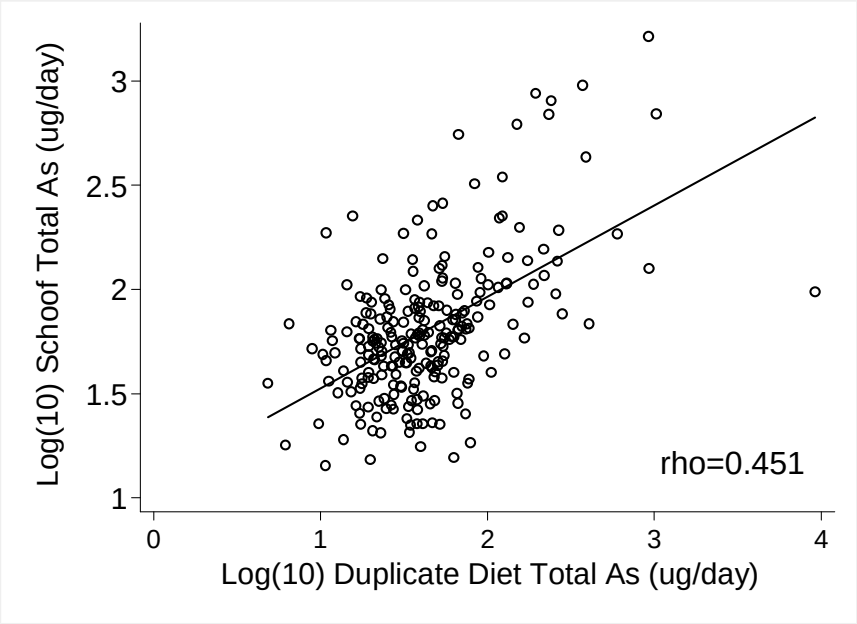


Figure 8.1. Scatterplots and intraclass correlation between measured and modeled dietary total As exposures: a) Duplicate diet vs. TDS max, and b) Duplicate diet vs. Schoof total.

Crude models

Crude models of the relation between urinary total As and dietary As (log-transformed) are shown separately for ABS and NHEXAS in Table 8.3. Duplicate diet As was a significant predictor of urinary As above vs. below the LOD in ABS (OR=4.20, 95% CI, 1.19-14.82), but not in NHEXAS (OR=1.94, 95% CI, 0.74-5.11). In the restricted model limited to subjects with urinary As above the LOD, duplicate diet, TDS mean, TDS max and Schoof total As were statistically significant linear predictors of urinary As in NHEXAS (n~102), but in ABS (n~36), none of the dietary As estimates were significant. Based on R-squared values from the restricted simple linear models, duplicate diet As accounted for approximately 17% of the variance in log(10) urinary As in NHEXAS participants with measurable urinary As. TDS max accounted for approximately 12.0%, Schoof total and TDS mean for 11 and 10%, respectively, and Schoof inorganic As for approximately 2% of the total variance in urinary As. The effects of dietary As on urinary As were similar in the crude and adjusted models for each study population.

Table 8.3. Two-stage simple regression analysis of measured and modeled dietary As intake and urinary As excretion in NHEXAS-AZ and ABS: 1) Stage 1, unrestricted logistic regression of urine total As (above vs. below the LOD); 2) Stage 2, restricted linear regression models using bootstrap sampling of urine total As as a continuously-distributed variable, restricted to subjects with urinary As above the LOD.

1) Stage 1 Models	NHEXAS-AZ			ABS		
	N	OR (95% CI)	*P*-value	N	OR (95% CI)	*P*-value
Dup Diet total As	155	1.94 (0.74-5.11)	0.178	85	4.20 (1.19-14.82)	0.026
TDS total mean As	166	1.34 (0.76-2.39)	0.310	86	1.25 (0.74-2.13)	0.406
TDS total maximum As	166	1.71 (0.62-4.69)	0.300	86	1.57 (0.68-3.61)	0.285
Schoof total As	166	1.95 (0.49-7.83)	0.344	86	1.85 (0.70-4.92)	0.215
Schoof inorganic As	166	0.96 (0.25-3.76)	0.955	86	0.84 (0.259-2.85)	0.775

2) Stage 2 Models	NHEXAS-AZ				ABS			
	N	Coefficient ± s.e.	R^2	*P*-value	N	Coefficient ± s.e.	R^2	*P*-value
Dup Diet total As	102	0.346±0.107	0.167	0.001	36	0.306±0.215	0.080	0.154
TDS total mean As	106	0.208±0.065	0.104	0.002	37	0.069±0.084	0.021	0.407
TDS total maximum As	106	0.368±0.123	0.120	0.003	37	0.117±0.128	0.026	0.364
Schoof total As	106	0.521±0.176	0.106	0.003	37	0.018±0.188	0.000	0.923
Schoof inorganic As	106	0.229±0.172	0.019	0.184	37	-0.337±0.196	0.073	0.091

Adjusted models

Two-stage multivariable regression models of urinary As in the combined population, are shown in Table 8.4. In the unrestricted models, duplicate diet was of borderline statistical significance after adjustment (OR=2.18, *P*=0.053), and TDS mean and TDS max approached significance (both *P*<0.080). In the restricted models, duplicate diet, TDS total, TDS max, and Schoof total As were statistically significant linear predictors of urinary As. None of the covariates in either the first or second stage regression models were statistically significant.

In models stratified by Hispanic ethnicity, the results of the unrestricted analyses for ABS and NHEXAS were similar to those in the unstratified models, i.e., mostly non-significant. There were too few non-Hispanic subjects in ABS with urinary As > LOD to run the restricted analyses (n=4). In NHEXAS, Hispanics (n=28) showed statistically significant relationships between TDS mean, TDS max, and Schoof total dietary As and urinary As in the restricted analyses (all *P*-values < 0.009), and non-Hispanics (n=73) showed a significant relationship only between duplicate diet As and urinary As (*P*=0.010).

Sensitivity analyses

Seafood consumption is directly related to urinary total As and dietary As. Potential confounding by seafood consumption was addressed in several ways. In the combined population, 34 (13%) subjects reported eating seafood, 25 of whom had measureable urinary As. In both unrestricted and restricted crude models, seafood consumption was a significant predictor of urinary As (P=0.024 and 0.001, respectively) (data not shown). In the restricted model that included both duplicate diet As and seafood consumption as covariates, both were statistically significant. When seafood consumption was included in TDS and Schoof As exposure models, neither estimate of total dietary As was statistically significant--seafood consumption alone predicted urinary total As. Models that excluded seafood eaters did not have sufficient power.

Analyses stratified by tap water As concentration

Drinking and cooking water As were not predictive of urinary As in any of the models. Models stratified by tap water As concentration were run on the combined population to determine whether the relationship between dietary and urinary As would vary depending on exposure to tap water As

above versus below cut points of 10 ppb (the EPA MCL) and 5 ppb. In the combined population, only 41 (16%) subjects lived in homes with tap water As concentration > 10 ppb. However, the relation between dietary As and urinary As was similar, regardless of stratification. Stratified analyses were also run with a tap water As cut point of 5 ppb. In those subjects with water As ≤ 5 ppb, duplicate diet As was associated with 3.75 times increased odds (95% CI, 1.21-11.57) of having urinary As above the LOD, and, in the restricted model, showed a significant relationship to urinary As (similar to that in unstratified analyses).

Table 8.4. Two-stage mixed model regression analysis of measured and modeled dietary As intake and urinary total As excretion in the combined population, adjusted for sex, ethnicity, current smoking, As exposure from drinking water and cooking water: 1) Stage 1, unrestricted linearized mixed models using bootstrap sampling of urine arsenic (above vs. below the LOD); 2) Stage 2, restricted linear mixed models of urinary total arsenic as a continuously-distributed variable, restricted to subjects with urinary As above the LOD.

1) Stage 1 Models	COMBINED POPULATION		
	N	OR (95% CI)	*P*-value
†Dup Diet total As	227	2.18 (0.99-4.80)	0.053
TDS total mean As	223	1.47 (0.97-2.25)	0.071
TDS total maximum As	223	1.85 (0.93-3.68)	0.077
Schoof total As	223	1.70 (0.73-3.96)	0.221
Schoof inorganic As	223	0.51 (0.20-1.33)	0.170

2) Stage 2 Models	COMBINED POPULATION		
	N	Coefficient ± s.e.	*P*-value
†Dup Diet total As	132	0.338±0.086	0.001
TDS mean total As	130	0.154±0.061	0.011
TDS max total As	130	0.277±0.101	0.006
Schoof total As	130	0.286±0.138	0.038
Schoof inorganic As	130	0.038±0.132	0.776

†As in water used for food preparation was incorporated in the analytic sample matrix and not included as a covariate in the duplicate models.

4. Discussion

Archived laboratory and questionnaire data on 252 participants in the Arizona Border Survey and the National Health Exposure Assessment Survey-Arizona were used in this study to compare measured and modeled dietary As exposure. Modeled dietary total As intake based on TDS maximum concentrations yielded estimates of exposure similar to the measured As in the duplicate diets (geometric means approximately 34 µg/day in NHEXAS, and 68 µg/day in ABS). The TDS maximum residue values yielded the best estimates of central tendency and were more highly correlated with the measured duplicate diet than modeled estimates based on other data sources. Estimates based on Schoof et al. (1999) were highly correlated with those based on TDS max As residues, and also moderately well correlated with measured exposure--though Schoof estimates were markedly higher than measured. In analyses restricted to the 57% of subjects in the combined population with measurable urinary As excretion, dietary total As based on duplicate diet and all of the modeled estimates were statistically significant linear predictors of urinary total As, albeit not all of the modeled estimates are equally accurate.

Duplicate diet As was markedly higher in the ABS population as compared to NHEXAS-AZ, and in Hispanics as compared to non-Hispanics, and these trends were reflected in the median estimates based on TDS max and Schoof, but not by the TDS mean estimates. The median dietary total As exposures modeled from TDS means were also not correlated with measured As intake and appear to grossly under-estimate exposure, suggesting that maximum estimates or modeled estimates based on foods that contribute approximately 90% of dietary intake (Schoof et al., 1999) more accurately reflect measured exposure than do the mean As residues for all foods.

Measured total As concentration from individual water samples collected from household sources and self-reported consumption data enabled person-specific modeling of water As intake. Neither drinking water nor cooking water was significantly associated with urinary As in crude or adjusted models. Models stratified by tap water As concentration above vs. below 10 ppb and 5 ppb showed a positive linear relationship between dietary As (measured and modeled) and urinary As, regardless of stratification. In this study population, total As exposure from food was approximately 14 times greater than that from drinking and

cooking water combined, and the effects of tap water As concentration above vs. below the MCL on urinary As were negligible.

Despite similar study designs and methods, there were marked differences in demographic characteristics and the distribution of dietary, water and urinary As between the NHEXAS-AZ and ABS studies. ABS was older, had more females, more Hispanics, and fewer smokers than NHEXAS. Although ABS subjects did not collect beverage samples, duplicate diet collection protocols were the same in both studies, and beverages were listed in the diaries. Multiple water samples were collected in both studies and incorporated in the analysis. In NHEXAS, kilocalorie intake, based on the diaries, was on average twice that of ABS. Nevertheless, ABS had markedly higher levels of measured and modeled dietary As than NHEXAS, with the exception of TDS mean As, which was lower in ABS. The differences between the study populations could not be accounted for by discrepancies in data collection protocols, nor were they related to population differences in consumption of seafood, ethnicity, sex, body weight, body mass index (BMI) or age. It is likely that certain foods that tend to be high in As, such as rice, chicken,

tortillas and beans, are preferentially eaten along the Arizona-Mexico border.

B. Specific Aim 2. *To evaluate the contribution of modeled dietary total and inorganic arsenic intake and water arsenic on total and speciated urinary arsenic in Hispanic and non-Hispanic Arizonans in the Binational Arsenic Exposure Study (BAsES). The effects of dietary nutrient intake on arsenic methylation and effect modification by exposure to household water As concentrations above versus below the MCL (10 ppb) will be evaluated.*

1. Rationale

The objectives of this aim are to model the effects of dietary total and inorganic arsenic intake on methylation of urinary arsenic in a study of arsenic exposure in four Arizona communities with regionally high and low levels of natural arsenic contamination in the ground water. The effects of dietary As intake on arsenic methylation, in addition to the effects of drinking and cooking water As, will enhance our understanding of the role of dietary intake in overall exposure and of the need for regulation of dietary As for the protection of public health. Furthermore, the effects of dietary intake of specific

nutrients on methylation efficiency will be evaluated. The impact of potential confounders on the relation between dietary As intake and urinary As biomarkers, including drinking and cooking water As, sex, BMI, age, and current smoking, will also be addressed.

2. Study-specific Methods

The population studied in Aim 2, BAsES-Arizona, is described in detail in the section on "Overall Approach" in "PRESENT STUDY." Total and inorganic dietary As were modeled from 24-hour dietary recall interviews using the Schoof total and inorganic As residue data. The effects of dietary total and inorganic arsenic on urinary As biomarkers (total, sum of species, %MMA and DMA:MMA) were evaluated separately in crude and adjusted models. As described in Appendix H, urinary arsenic concentrations were adjusted for specific gravity (Nermell et al., 2008), but analyses were performed on both unadjusted and adjusted concentrations. Urinary total and sum of species arsenic, dietary total and inorganic arsenic, and drinking and cooking water arsenic values were log-transformed to normalize the distributions for analysis.

Multilevel mixed-effects linear regression models were used to account for covariance within study site (community) and within households. These models directly estimated all variances and covariances of the random effects (unstructured covariance). Models were fitted using maximum likelihood estimates. Restricted models that included only those subjects who did not report eating seafood were compared to models with all subjects.

3. Results

Population characteristics and exposures

Population characteristics for the Arizona BAsES study are shown in Table 8.5 by ethnic group. Approximately 55% of the 223 subjects were female, with similar proportions of females in both ethnic groups. Except in the community of New River, where only 1% of subjects self-identified as Hispanic, between 33-39% of subjects from the other study sites were Hispanic white, and the rest self-identified as non-Hispanic white. The mean age of the population was 55.6 ± 15.0 years; Hispanics were an average of 9 years younger than non-Hispanics. Mean body mass index (BMI) was 31.3 in Hispanics and 28.5 in non-Hispanics.

Table 8.5. Characteristics of the Arizona BAsES study population by ethnicity.

	Total Population	Non-Hispanic White	Hispanic White	*P*-value[‡]
Subjects, n(%)	223	171(76.68)	52(23.32)	
Female, n(%)	123(55.16)	95(55.56)	28(53.85)	0.828
Study site, n(%)				
Ajo	31(13.90)	19(61.29)	12(38.71)	
New River	76(34.08)	75(98.68)	1(1.32)	0.001
San Manuel	49(21.97)	33(67.35)	16(32.65)	
Tucson	67(30.04)	44(65.67)	23(34.33)	
Age (yrs), mean ± s.d.	55.56 ± 15.02	57.68 ± 14.32	48.61 ± 15.30	0.001
BMI, mean ± s.d	29.12 ± 6.39	28.46 ± 6.24	31.27 ± 6.46	0.005
Current smoker, n(%)	33(14.80)	30(17.54)	3(5.77)	0.036
Drinking water As, geo mean (95% CI)				
concentration[§] (µg/L)	7.49 (6.3-8.9)	8.85(7.1-11.0)	4.42(3.6-5.4)	0.001
exposure[§] (µg/day)	4.91 (3.8-6.3)	5.58(4.1-7.7)	3.32(2.3-4.7)	0.086
Cooking water As, geo mean (95% CI)				
exposure[§] (µg/day)	2.36(1.8-3.1)	2.49(1.8-3.5)	2.05(1.3-3.1)	0.541

Table 8.5 (continued)

	Total Population	Non-Hispanic White	Hispanic White	*P*-value‡
Dietary As[1] geo mean (95% CI),(µg/day)				
Total[1]	32.8(29.3-36.6)	31.8(28.0-36.1)	35.4(28.0-44.8)	0.418
Inorganic[1]	5.9(5.5-6.4)	5.8 (5.3-6.3)	6.2(5.1-7.7)	0.438
Urinary arsenic (µg/L), unadjusted, geo means (95% CI)				
Total	25.6(22.5-29.2)	27.8(23.7-32.7)	19.9(16.5-24.0)	0.034
As^{+3}	0.55(0.5-0.6)	0.60(0.5-0.7)	0.43(0.3-0.6)	0.078
As^{+5}	0.48(0.4-0.6)	0.51(0.4-0.6)	0.38(0.3-0.5)	0.144
MMA	1.24(1.1-1.4)	1.37(1.1-1.6)	0.88(0.7-1.1)	0.010
DMA	8.05(7.1-9.1)	8.66(7.4-10.1)	6.40(5.4-7.6)	0.045
Arsenobetaine	1.75(1.3-2.3)	2.02(1.4-2.9)	1.14(0.7-1.8)	0.101
Sum of species	10.85(9.6-12.3)	11.65(10.0-13.6)	8.61(7.2-10.2)	0.048
Urinary arsenic (µg/L), adjusted[2], geo means (95% CI)				
Total	30.9(27.5-34.7)	33.1(28.6-38.3)	24.7(21.8-28.1)	0.037
As^{+3}	0.66(0.6-0.8)	0.71(0.6-0.8)	0.54(0.4-0.7)	0.102
As^{+5}	0.57(0.5-0.7)	0.60(0.5-0.7)	0.47(0.3-0.6)	0.231
MMA	1.48(1.3-1.7)	1.63(1.4-1.9)	1.10(0.9-1.3)	0.016
DMA	9.7(8.6-10.9)	10.3(8.9-11.9)	7.9(6.7-9.4)	0.070

Table 8.5 (continued)

	Total Population	Non-Hispanic White	Hispanic White	*P*-value[‡]
Arsenobetaine	2.1(1.6-2.8)	2.4(1.7-3.4)	1.4(0.9-2.3)	0.116
Sum of species	13.0(11.6-14.7)	13.8(11.9-16.1)	10.7(9.2-12.5)	0.073
%MMA	11.4(10.8-12.0)	11.7(11.0-12.5)	10.2(9.2-11.4)	0.033
DMA:MMA	6.5(6.1-6.9)	6.3(5.9-6.8)	7.2(6.3-8.3)	0.078
Urine specific gravity, mean (95% CI)				
	1.015(1.01-1.02)	1.016(1.01-1.02)	1.015(1.01-1.02)	0.339

[§] Weighted values; [1] Dietary As adjusted for caloric extremes; [2] Values adjusted for specific gravity; [‡] *P*-values based on chi-square test and analysis of variance

There were no differences in exposure to dietary total or inorganic As between ethnic groups. Mean dietary total As exposure was 32.8 µg/day, mean dietary inorganic As was 5.9 µg/day. In comparison, mean exposure to drinking water As (based on concentration of As in sources used, frequency of use of each source, and total volume) was 4.9 µg/day (95% CI, 3.8-6.3), and mean exposure to cooking water As (based on the concentration of As in sources used, frequency of use, and volume added in food home preparation) was 2.36 µg/day (95% CI, 1.8-3.1). Exposure to As in diet and water did not vary by ethnic group. Arsenic concentration in water used for drinking was significantly higher in the non-Hispanic than in the Hispanic subpopulation (geometric mean 8.85 vs. 4.42, respectively, $P<0.001$).

Overall, urinary As biomarkers adjusted for specific gravity had higher geometric means than the unadjusted values, but the relation to ethnicity was consistent regardless of adjustment. Hispanics had lower mean concentrations of all urinary biomarkers. MMA and %MMA were significantly higher in non-Hispanics. Percent MMA varied between 3.7 to 41% in the BAsES population, with a geometric mean of 12.3%. The geometric mean ratio of

DMA:MMA was slightly higher among Hispanics, 7.2 vs. 6.3, but this difference was not significant (P=0.078).

Crude models

Univariate models of urinary total As, sum of species As, %MMA and DMA:MMA are shown in Table 8.6. Dietary total As was a significant predictor of urinary total and sum of species As (both P<0.001), but not of %MMA or the ratio of DMA:MMA excreted. Exposure to As from drinking water and water used for cooking were also predictors of both urinary total and sum of species As (P<0.001). Dietary inorganic As intake was positively associated with sum of species As (P<0.001), but with no other urinary As biomarkers. Hispanic ethnicity was associated with lower levels of urinary total As and %MMA, as compared with non-Hispanics. In the crude models, BMI was inversely related to urinary total As, sum of species As, and %MMA, and positively related to DMA:MMA (all P<0.001). Current smoking was a positive univariate predictor of total and sum of species As, and age and sex were not associated with any of the biomarkers.

Table 8.6. Univariate predictors of urinary arsenic biomarkers based on linear mixed model regression in BAsES.

	Urinary Total As		Sum of species As	
	β(S.E.)	*P*	β(S.E.)	*P*
Dietary total As (µg/day)*	0.276(0.062)	0.001	0.269(0.067)	0.001
Dietary inorganic As (µg/day)*	0.108(0.086)	0.212	0.358(0.090)	0.001
Drinking water As (µg/day)*	0.174(0.029)	0.001	0.247(0.028)	0.001
Cooking water As (µg/day)*	0.136(0.029)	0.001	0.234(0.039)	0.001
Sex (female)	0.013(0.036)	0.726	0.000(0.040)	0.994
Age	0.002(0.002)	0.298	0.002(0.002)	0.349
BMI	0.008(0.004)	0.023	-0.014(0.004)	0.001
Hispanic	0.136(0.061)	0.025	-0.107(0.065)	0.098
Current smoking	0.147(0.060)	0.015	0.136(0.066)	0.040

Table 8.6 (continued)

	% MMA		DMA:MMA	
	β(S.E.)	*P*	β(S.E.)	*P*
Dietary total As (µg/day)*	-0.014(0.009)	0.117	1.380(0.744)	0.064
Dietary inorganic As (µg/day)*	-0.000(0.012)	0.998	-0.900(0.004)	0.370
Drinking water As (µg/day)*	0.007(0.004)	0.101	-0.237(0.334)	0.477
Cooking water As (µg/day)*	0.005(0.004)	0.262	-0.268(0.347)	0.441
Sex (female)	-0.008(0.006)	0.152	0.537(0.542)	0.322
Age	0.000(0.000)	0.745	-0.001(0.018)	0.976
BMI	-0.003(0.000)	0.001	0.234(0.040)	0.001
Hispanic	-0.017(0.008)	0.034	0.944(0.640)	0.140
Current smoking	0.009(0.009)	0.312	-0.055(0.764)	0.943

* Values log(10)-transformed.

Adjusted models

Multivariable models yielded similar results: Dietary total As, adjusted for BMI, sex, age, ethnicity, current smoking, drinking and cooking water As, was a significant predictor of urinary total and sum of species As (Table 8.7). The urinary sum of species As model was the most parsimonious model, based on Akaike's Information Criterion, and the best-fitting model (adjusted R^2 = 0.438). The only statistically significant predictors of %MMA and DMA:MMA were female sex and BMI (both negatively associated with %MMA and positively associated with DMA:MMA). Dietary total As approached significance in the models of %MMA (*P*=0.109) and DMA:MMA (*P*=0.088), but neither drinking nor cooking water were predictive.

Effects of dietary inorganic As on urinary As were also assessed in multivariable models. Inorganic As from food was associated with urinary sum of species As (Table 8.8), but not with any other urinary biomarkers (not shown), and drinking water As, cooking water As, BMI, and current smoking were significant confounders in this model (adjusted R^2=0.426).

Table 8.7. Multivariable linear mixed models: Effect of dietary total arsenic on urinary As biomarkers in BAsES.

	Urinary Total As		Urinary Sum of Species As	
	β(SE)	*P*	β(SE)	*P*
Dietary total As*	0.259(0.059)	0.001	0.237(0.056)	0.001
Drinking water As*	0.133(0.033)	0.001	0.167(0.031)	0.001
Cooking water As*	0.043(0.032)	0.175	0.124(0.031)	0.001
Sex (female)	0.029(0.034)	0.400	0.038(0.035)	0.285
Age	0.002(0.002)	0.133	0.002(0.001)	0.124
BMI	-0.008(0.003)	0.019	-0.012(0.003)	0.001
Hispanic	-0.070(0.055)	0.203	-0.016(0.052)	0.751
Current smoking	0.150(0.057)	0.008	0.143(0.056)	0.010
R^2	0.292		0.459	
Adjusted R^2	0.265		0.438	

Table 8.7 (continued)

	Urinary %MMA		Urinary DMA:MMA	
	β (SE)	*P*	β (SE)	*P*
Dietary total As*	-0.014(0.008)	0.109	1.220(0.715)	0.088
Drinking water As*	0.004(0.005)	0.400	0.084(0.387)	0.829
Cooking water As*	0.001(0.005)	0.739	-0.197(0.395)	0.618
Sex (female)	-0.013(0.005)	0.016	1.10(0.515)	0.033
Age	-0.000(0.000)	0.807	0.012(0.018)	0.511
BMI	-0.003(0.000)	0.001	0.231(0.041)	0.001
Hispanic	-0.007(0.008)	0.384	0.412(0.642)	0.521
Current smoking	0.011(0.008)	0.208	-0.150(0.746)	0.841
R^2	0.227		0.163	
Adjusted R^2	0.198		0.131	

* Values log(10)-transformed

Table 8.8. Multivariable model of effect of dietary inorganic arsenic on urinary As sum of species in BAsES.

	Urinary Sum of Species As	
	β(SE)	*P*
Dietary inorganic As*	0.265(0.077)	0.001
Drinking water As*	0.169(0.031)	0.001
Cooking water As*	0.117(0.031)	0.001
Sex (female)	0.028(0.036)	0.434
Age	0.002(0.001)	0.257
BMI	-0.012(0.003)	0.001
Hispanic	-0.015(0.052)	0.770
Current smoking	0.164(0.056)	0.004
R^2	0.447	
Adjusted R^2	0.426	

* Values log(10)-transformed

Conditional analysis: Non-seafood eaters

In conditional analyses restricted to subjects who did not report eating any fish or shell fish (n=191), the relation between dietary total As and urinary sum of species As did not change (Figure 8.2 a and b). There was, however, no relation in the models conditioned on no seafood consumption between dietary As and urinary total As or As methylation (results not shown).

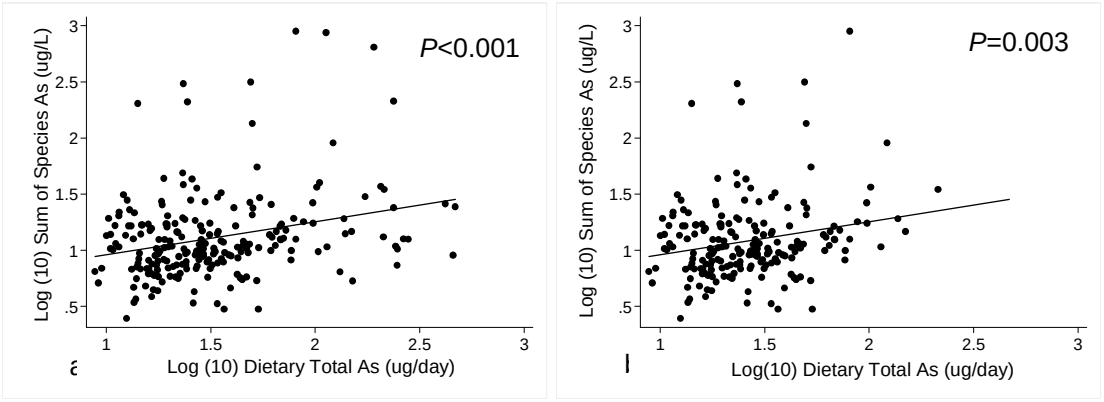


Figure 8.2. Regression of urinary sum of species As over dietary total As in the total population (a) and in non-seafood eaters only (b), BAsES.

Analyses stratified by tap water As concentration

In analyses stratified by water As concentrations >10 ppb versus ≤10 ppb (the MCL), there were marked differences in the proportion of total As intake attributable to ingestion of food versus ingestion of water (Table 8.9). Total ingested As was 165% higher for total As and 285% higher for inorganic As in subjects with high household water arsenic. In subjects living in households with ≤ 10 ppb tap water As, dietary total As composed almost 87% of total As ingested and dietary inorganic As composed 54% of total inorganic As ingested. Drinking water and water used in food preparation ("cooking") contributed a much higher proportion of total exposure in subjects living in households with water As above the MCL. In contrast, food contributed only about 60% of total As and 21% of inorganic As intake in subjects living in households with tap water As >10 ppb.

Models stratified by tap water As concentration are shown in Tables 8.10 (dietary total As models) and 8.11 (dietary inorganic As models). Dietary total As was a significant predictor of urinary total and urinary sum of species As, regardless of stratification, but drinking water As exposure was not a significant predictor of urinary total

As in the lower stratum. In the models of urinary sum of species As, there was no interaction between dietary As intake and household tap water strata—the effects of dietary and drinking water As were similar and statistically significant in both strata. Dietary inorganic As was not a significant predictor of urinary total As, regardless of stratification, but was a significant predictor of urinary sum of species As in households with water As concentrations below the MCL. In the dietary inorganic As models of sum of species As, drinking water As was significant in both strata, but cooking water As was only significant in households with tap water concentrations > 10ppb.

There was no interaction between diet and household water As strata observed in models of %MMA or DMA:MMA (not shown).

Table 8.9. Geometric mean dietary, drinking water and cooking water As (µg/day) and percent of total ingested, stratified by household water As concentration, BAsES.

	Household Water As ≤ 10 ppb (n=161)	Household Water As > 10 ppb (n=62)
	µg/day (%)	µg/day (%)
Dietary total As	31.55 (86.70)	36.18 (60.35)
Drinking water As	3.21 (8.82)	16.30 (27.19)
Cooking water As	1.63 (4.48)	7.47 (12.46)
TOTAL As INTAKE	***36.39***	***59.95***
Dietary inorganic As	5.75 (54.30)	6.44 (21.32)
Drinking water As	3.21 (30.31)	16.30 (53.95)
Cooking water As	1.63 (15.39)	7.47 (24.73)
TOTAL INORGANIC As INTAKE	***10.59***	***30.21***

Table 8.10. Stratified models: Mixed model regression of urinary total As and sum of species As on dietary total As, stratified by household water arsenic concentration ≤ vs. > 10 ppb, BAsES.

	Household Water As ≤10 ppb (n=160)		Household Water As >10 ppb (n=60)	
	β (SE)	*P*	β (SE)	*P*
Urinary Total As				
Dietary total As*	0.137(0.059)	0.020	0.539(0.129)	0.001
Drinking water As*	0.052(0.038)	0.176	0.169(0.062)	0.006
Cooking water As*	0.003(0.035)	0.928	0.045(0.062)	0.467
Sex (female)	-0.036(0.035)	0.301	0.140(0.076)	0.066
Age	0.001(0.001)	0.552	0.009(0.004)	0.027
BMI	-0.006(0.003)	0.084	-0.005(0.007)	0.502
Hispanic	-0.028(0.050)	0.570	-0.604(0.265)	0.023
Current smoking	0.143(0.058)	0.014	0.171(0.115)	0.137

Table 8.10 (continued)

	Household Water As ≤10 ppb (n=160)		Household Water As >10 ppb (n=60)	
	β (SE)	*P*	β (SE)	*P*
Urinary Sum of Species As				
Dietary total As*	0.191(0.053)	0.001	0.307(0.128)	0.017
Drinking water As*	0.080(0.036)	0.025	0.223(0.060)	0.001
Cooking water As*	0.054(0.032)	0.096	0.165(0.062)	0.008
Sex (female)	-0.005(0.036)	0.882	0.099(0.078)	0.207
Age	0.001(0.001)	0.537	0.007(0.004)	0.069
BMI	-0.009(0.003)	0.002	-0.012(0.007)	0.092
Hispanic	0.007(0.044)	0.873	-0.442(0.258)	0.087
Current smoking	0.087(0.056)	0.117	0.279(0.117)	0.017

* Values log(10)-transformed

Table 8.11. Stratified models: Mixed model regression of urinary total As and sum of species As on dietary inorganic As, stratified by household water arsenic concentration ≤ vs. > 10 ppb, BAsES.

	Household Water As ≤10 ppb (n=160)		Household Water As >10 ppb (n=60)	
	β (SE)	*P*	β (SE)	*P*
Urinary Total As				
Dietary inorganic As*	0.007(0.077)	0.931	0.260(0.214)	0.225
Drinking water As*	0.053(0.039)	0.172	0.196(0.069)	0.005
Cooking water As*	0.016(0.036)	0.663	0.032(0.070)	0.652
Sex (female)	-0.050(0.035)	0.155	0.164(0.087)	0.059
Age	0.000(0.001)	0.798	0.008(0.004)	0.067
BMI	-0.005(0.003)	0.130	-0.002(0.008)	0.756
Hispanic	-0.025(0.050)	0.620	-0.550(0.296)	0.063
Current smoking	0.151(0.059)	0.011	0.197(0.130)	0.130

Table 8.11 (continued)

	Household Water As ≤10 ppb (n=160)		Household Water As >10 ppb (n=60)	
	β (SE)	*P*	β (SE)	*P*
Urinary Sum of Species As				
Dietary inorganic As*	0.281(0.068)	0.001	0.286(0.195)	0.142
Drinking water As*	0.081(0.035)	0.022	0.233(0.062)	0.001
Cooking water As*	0.036(0.032)	0.269	0.151(0.064)	0.018
Sex (female)	-0.021(0.036)	0.568	0.121(0.081)	0.134
Age	0.000(0.001)	0.877	0.007(0.004)	0.091
BMI	-0.009(0.003)	0.002	-0.011(0.008)	0.133
Hispanic	0.007(0.043)	0.877	-0.429(0.266)	0.107
Current smoking	0.109(0.055)	0.047	0.295(0.120)	0.014

* Values log(10)-transformed

Nutrient models

The effect of specific dietary nutrient intake on urinary As biomarkers was tested, first by evaluating the relation between single nutrients and each biomarker. The nutrients initially evaluated included total folate, Vitamin B-12, Vitamin B-6, methionine, and protein. Dietary nutrients, adjusted for extreme energy reporting, were log-transformed to normalize the distributions for analysis. Scatterplots of the relation between several individual nutrients and urinary As biomarkers are shown in Figures 8.3 and 8.4. The negative association between protein intake and %MMA (Figure 8.3d) and positive association between protein intake and DMA:MMA (Figure 8.4d)were statistically significant (P=0.004 and 0.001, respectively). Methionine and protein intake were very highly correlated (r=0.965, P<0.001) and showed the same relationship with %MMA and DMA:MMA. Because of its high correlation with total protein intake, methionine was dropped from the multivariable nutrient models. Dietary Vitamin B-12 was significantly negatively correlated with DMA:MMA (P=0.013). Vitamin B-6 and Vitamin B-12 intake showed borderline

significance in relation to %MMA in simple mixed model regression analysis (*P*-values <0.10).

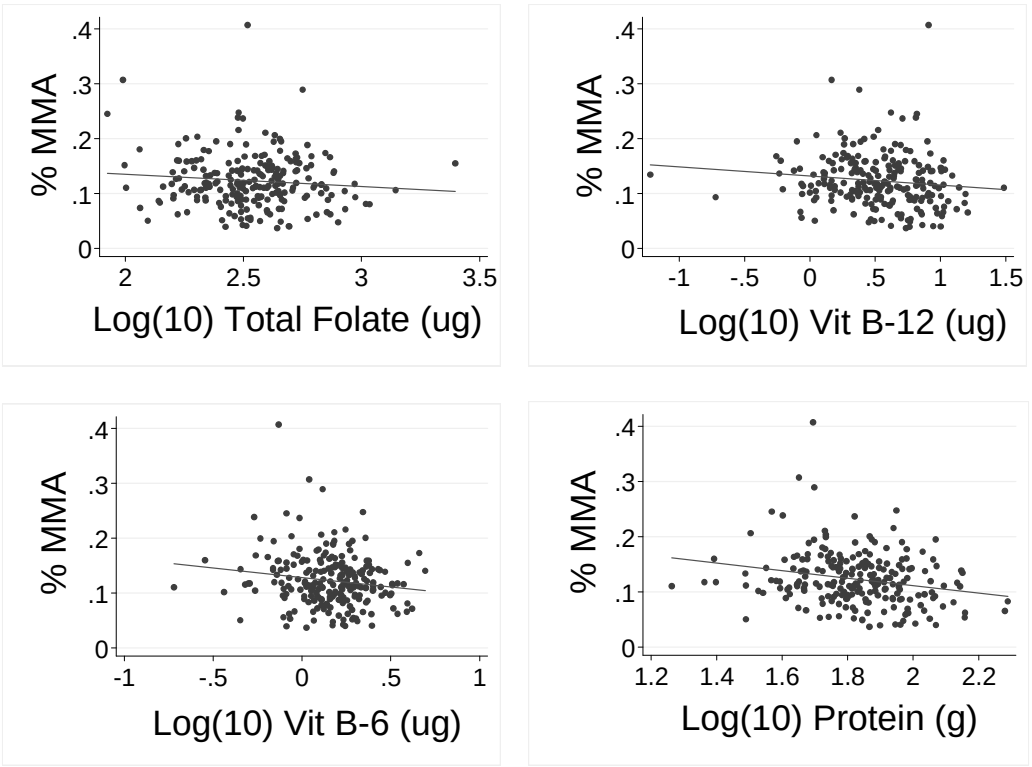


Figure 8.3. Scatterplots of %MMA over: dietary a) total folate (*P*=0.111), b) Vitamin B-12 (*P*=0.095), c) Vitamin B-6 (*P*=0.074) and d) total protein (*P*=0.004) in BAsES.

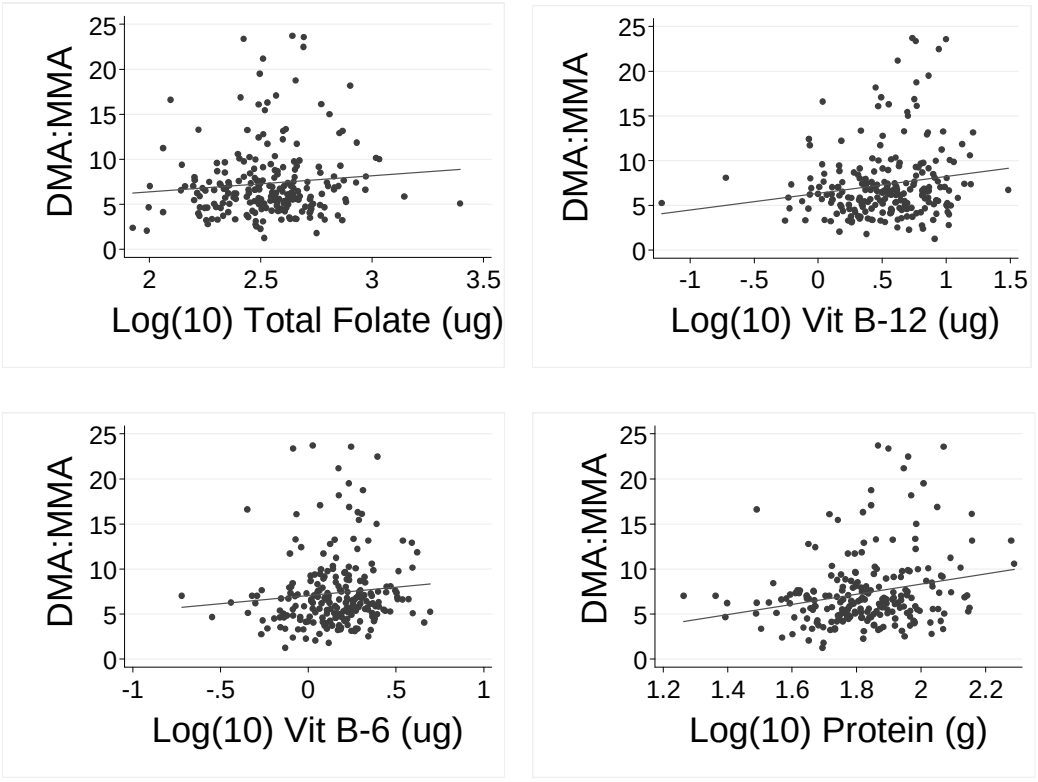


Figure 8.4. Scatterplots of DMA:MMA over: a) dietary total folate (*P*=0.150), b) Vitamin B-12 (*P*=0.013), c) Vitamin B-6 (*P*=0.138), and d) protein intake (*P*=0.001), BAsES.

When total folate, Vitamin B-12, Vitamin B-6, and protein were entered together into the adjusted models, protein was a significant negative predictor of urinary sum of species As (Table 8.12), and the full model explained 48% of the total variance in urinary sum of species As. Protein intake was also a positive predictor of dietary total As.

Table 8.12. Nutrient models: Effect of dietary total As and dietary nutrients on urinary sum of species As and DMA:MMA in BAsES.

	Urinary Sum of Species As*		DMA:MMA	
	β(SE)	*P*	β(SE)	*P*
Dietary protein*	-0.525(0.174)	0.003	3.661(1.289)	0.110
Dietary folate*	-0.037(0.099)	0.708	1.103(1.335)	0.367
Dietary Vit B-12*	-0.009(0.061)	0.879	0.772(0.818)	0.345
Dietary Vit B-6*	0.185(0.125)	0.139	-1.365(2.289)	0.404
Dietary total As*	0.285(0.059)	0.001	0.760(0.748)	0.309
Drinking water As*	0.177(0.031)	0.001	0.019(0.385)	0.961
Cooking water As*	0.111(0.031)	0.001	-0.111(0.394)	0.779
Sex (female)	0.012(0.037)	0.741	1.403(0.535)	0.009
Age	0.002(0.001)	0.246	0.016(0.018)	0.369
BMI	-0.009(0.003)	0.009	0.201(0.043)	0.001
Hispanic	-0.031(0.051)	0.542	0.518(0.638)	0.417
Current smoking	0.146(0.055)	0.008	0.174(0.740)	0.815
R^2	0.483		0.190	
Adjusted R^2	0.454		0.143	

* Log(10)-transformed values

4. Discussion

In a randomly selected population of 223 Hispanic and non-Hispanic whites residing in four communities in Arizona selected to represent communities with varying levels of As in their municipal water supply, dietary total arsenic was significantly associated with both urinary total arsenic excretion and sum of species As. Dietary inorganic As intake was also associated with urinary As sum of species, both before and after adjustment for covariates in the model. This study did not find a direct relationship between either dietary As or water As intake and patterns of methylation, i.e., effects on the percent of urinary As excreted as MMA or the ratio of DMA to MMA. In this study, Hispanics had lower concentrations of arsenic in their drinking water and in their urine, and there were no differences in intake of dietary As between Arizona Hispanics and non-Hispanics.

In models stratified by household water As above vs. below the EPA's MCL, dietary As was the primary contributor to total ingested As (both total and inorganic) in households with water As below the current MCL. Overall, an increase of 1 µg/day of estimated dietary total As had a

greater impact on urinary total and sum of species As than an increase of 1 µg/day of water As.

In a duplicate diet survey of dietary total arsenic exposure in 47 adult women in Bangladesh, Kile et al. (2007) found that water was the dominant exposure when drinking water As concentration was >50 ppb, and dietary As was the dominant exposure when water As concentrations were <50 ppb (Kile et al., 2007b). The median total As intake from food and drinking water in these women was estimated at 68 µg/day, with 48 µg/day from food only. In the Arizona BAsES population, the dominant exposure was from food, regardless of household water As concentration. In households with >10 ppb As, geometric mean total As ingested was 60 µg/day, 60% of which was from food, and in households with ≤10 ppb water As, mean ingested total As was 36 µg/day, 87% from food.

This study also found a reduction of total absorbed As dose with increased dietary intake of methionine and/or protein in adjusted models, and, in crude models, an increased rate of secondary methylation with greater intake of protein and Vitamin B-12. Inclusion of either protein or methionine intake in the models resulted in a

statistically significant reduction in urinary sum of species As in adjusted models. In a study of subjects residing in an area in the western U.S. with high water As concentrations, Steinmaus et al. (2005) reported similar findings--subjects in the lowest quartile of protein intake, as compared to the highest quartile, excreted a greater percent MMA.

C. Specific Aim 3. *To evaluate the contribution of modeled dietary total and inorganic arsenic intake and the effects of specific dietary nutrient intake on patterns of urinary arsenic methylation in a nationally-representative U.S. population sample from the 2003-2004 National Health and Nutrition Examination Survey (NHANES).*

1. Rationale

The objectives of Specific Aim 3 were to assess the effects of dietary arsenic and nutrient intake on urinary arsenic methylation patterns in the NHANES 2003-2004 as a way of comparing regional findings to a larger, independent national population sample. In NHANES, however, household water samples were not collected and subject-specific intake of drinking and cooking water arsenic could not be incorporated into the modeling. Nevertheless, the impact of dietary As and nutrient intake on As methylation could be evaluated taking into account the effects of racial/ethnic differences within the U.S. and the effects of sex, age, smoking status and BMI.

2. Study-specific Methods

The National Health and Nutrition Examination Surveys utilize a complex, multistage survey design and these require the use of sampling weights to account for differential probabilities of selection of sampled individuals, oversampling of selected populations, survey non-response, and post-stratification. The sampling weights are created in NHANES for use in analysis, and the weights are assumed to produce "unbiased" estimates that are representative of the U.S. Census civilian non-institutionalized population (CDC, 2011).

A one-third subsample of the NHANES 2003-2004 study population was selected by NHANES for analysis of urinary speciated arsenic. All statistical analyses utilized methods specific to analysis of survey data, and sampling weights specific to this NHANES subsample were applied. Linearization was used for calculating the standard errors of means, geometric means, and in simple and multiple linear regression analysis. Linear combinations were used for multiple comparisons of means among ethnic groups.

The methods previously used in analysis of urinary As in NHANES 2003-2004 to address values below the limit of

detection and to correct for variable water excretion rates by adjusting for creatinine were described in detail in Caldwell et al. (2009). Urinary As values below the LOD (0.6-1.2 µg/L) were assigned a value that was equal to the LOD divided by the square root of 2. Urinary As measures were log-transformed prior to statistical analyses. For measurements with >60% of values below the LOD, geometric means were not calculated.

Due to extensive debate regarding the effects of creatinine in modeling urinary As, statistical modeling was performed in three ways, using: 1)urinary As values unadjusted for creatinine, 2)urinary As values adjusted for creatinine, and 3)creatinine included as a separate covariate in the models, with and without creatinine interaction terms. The results of modeling unadjusted urinary As are presented in the next section, and the creatinine adjusted and creatinine covariate models are shown in Appendices I through L.

3. Results

Population characteristics and exposures

Overall characteristics of the NHANES 2003-2004 population subsample are shown in Table 8.13. There were 2420 observations with non-missing urinary As and dietary As measures. Non-Hispanic whites represented 71% of the total population, Non-Hispanic blacks, 11.7%, and Mexican-Americans, 8.7%. "Other Hispanics" and "other race" composed the remainder of the population sample (8.7%), and are assumed to represent ethnic admixtures. Mean age varied by ethnic group, ranging from 30 years (Mexican-Americans) to 41.6 years (non-Hispanic whites). About a quarter of the population sample reported current smoking, and most of these smokers were non-Hispanic white.

"Other" Hispanics also tended to have higher mean urinary As biomarkers than non-Hispanic whites. Box plots of urinary sum of species As, %MMA and DMA:MMA are shown by ethnic group in Figure 8.5.

Table 8.13. Population-proportional characteristics of NHANES 2003-04 by ethnic group.

	Total Sample	Non-Hispanic White	Mexican-American	Other Hispanic	Non-Hispanic Black	Other Race
Observations						
n	2420	1023	586	65	658	88
weighted %		71.0	8.7	3.7	11.7	5.0
Females						
n	1222	532	295	30	318	47
weighted %	52.0	37.2	4.3	1.6	6.4	2.5
Age in years						
geometric mean	39.0	41.6	30.2	34.1	34.5	31.3
95% CI	37.8-40.2	39.9-43.3	28.2-32.2	27.3-40.8	32.7-36.4	26.8-35.7
BMI						
geometric mean	27.0	27.0	26.8	25.5	28.5	27.3
95% CI	26.5-27.5	26.5-27.6	26.0-27.6	23.9-27.2	27.5-29.6	21.9-26.7
Current smoker						
n	303	174	43	5	71	10
weighted %	25.6	20.2	1.3	0.4	2.4	1.2

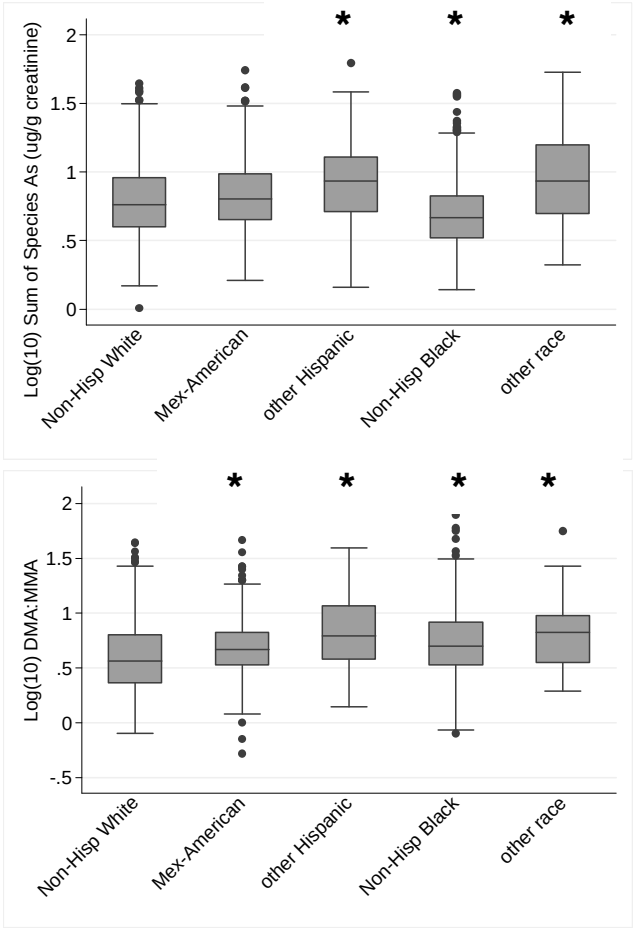


Figure 8.5. Urinary sum of species As and DMA:MMA by ethnic group. Asterisk indicates significant difference ($P<0.05$) in mean arsenic as compared with non-Hispanic whites (reference group).

Table 8.14. Urinary As biomarkers and dietary As intake in NHANES 2003-04 by ethnic group (population-proportional).

	Total Sample	Non-Hispanic White	Mexican-American	Other Hispanic	Non-Hispanic Black	Other Race	*P*-value
Urinary As, unadjusted[1] (µg/L)							
Total As							
gmean	8.29	7.14	9.12*	13.21*	11.79*	17.10*	<0.001
SE	1.07	1.07	1.06	1.12	1.09	1.21	
As^{+3}							
gmean	§	§	§	§	§	§	
As^{+5}							
gmean	§	§	§	§	§	§	
MMA							
gmean	§	§	§	§	§	§	
DMA							
gmean	3.68	3.27	4.66*	6.25*	4.33*	6.41*	<0.001
SE	1.05	1.05	1.04	1.13	1.06	1.10	
AsB							
gmean	1.54	1.36	1.16	2.37	2.35*	4.07*	0.002
SE	1.08	1.10	1.16	1.30	1.18	1.33	
Sum of species As							
gmean	6.52	5.97	7.62*	9.48*	7.10*	9.64*	<0.001
SE	1.04	1.03	1.04	1.09	1.04	1.08	

Table 8.14 (continued)

	Total Sample	Non-Hispanic White	Mexican-American	Other Hispanic	Non-Hispanic Black	Other Race	*P*-value
Urine creatinine (mg/dL)							
gmean	100.91	95.87	107.18*	96.12	140.41*	95.45	
SE	1.03	1.04	1.03	1.09	1.02	1.15	<0.001
Urinary As, adjusted (µg/g creatinine)							
Total As							
gmean	8.24	7.46	8.51	13.74*	8.39	17.91*	<0.001
SE	1.07	1.09	1.08	1.12	1.08	1.13	
As^{+3}							
gmean	§	§	§	§	§	§	
As^{+5}							
gmean	§	§	§	§	§	§	
MMA							
gmean	§	§	§	§	§	§	
DMA							
gmean	3.68	3.42	4.34*	6.50*	3.08	6.71*	<0.001
SE	1.06	1.07	1.07	1.10	1.06	1.10	
Arsenobetaine							
gmean	1.54	1.43	1.08*	2.47*	1.67	4.27*	0.001
SE	1.08	1.11	1.18	1.38	1.16	1.27	
Sum of species As							
gmean	6.47	6.25	7.11	9.87*	5.06*	10.09*	0.001
SE	1.05	1.06	1.07	1.08	1.04	1.10	

Table 8.14 (continued)

	Total Sample	Non-Hispanic White	Mexican-American	Other Hispanic	Non-Hispanic Black	Other Race	*P*-value
Dietary As[2] (µg/day)							
Total As							
gmean	41.08	37.3	41.0	70.1*	48.5*	79.6*	<0.001
SE	1.03	1.04	1.05	1.13	1.08	1.14	
Inorganic As							
gmean	6.20	5.82	6.49*	9.91*	6.19	10.33*	
SE	1.02	1.02	1.03	1.15	1.04	1.08	<0.001

[1]Values not adjusted for urine creatinine
[2]Values adjusted for caloric extremes. gmean, weighted geometric means; SE, linearized standard error
§ not calculated (>60% of values below the LOD)
P-values based on linear regression analysis and linear contrasts;
*Statistically significant difference (*P*<0.05), as compared with non-Hispanic whites.

Mean dietary total As was 41.1 (95% CI, 38.2-44.0) µg/day. Total dietary As intake was significantly higher among "other" Hispanics (70.1 µg/day), "other races" (79.6), and non-Hispanic blacks (48.5), as compared with non-Hispanic whites (37.3) (Figure 8.6). Mean dietary total As intake by Mexican-Americans was not significantly different from that of either non-Hispanic whites or blacks. There was no difference in dietary inorganic As intake between non-Hispanic whites and non-Hispanic blacks, but Mexican-Americans, other Hispanics, and other races had statistically higher intake than the other groups.

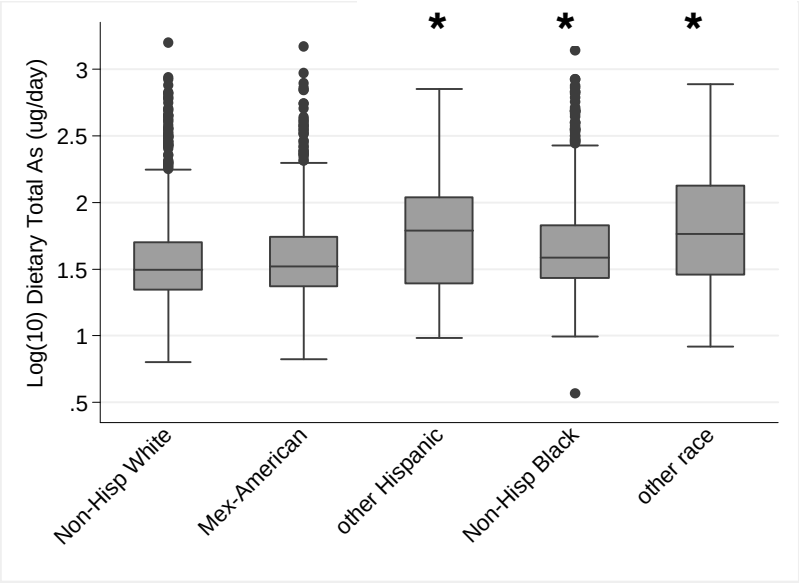


Figure 8.6. Box plot of dietary total As by ethnic group. Asterisk indicates significant difference ($P<0.05$) compared to non-Hispanic whites (reference group).

Crude models

Simple regression analysis of the relations between dietary total and inorganic As, consumption of seafood, urine creatinine, age, sex, BMI, current cigarette smoking and urinary As biomarkers (total As, sum of species As, %MMA, and DMA:MMA) are presented in Table 8.15. (The same analyses done with creatinine-adjusted urinary As biomarkers are shown in Appendix I.) Separately, dietary total and dietary inorganic As were statistically significant positive predictors of urinary total, sum of species arsenic, and DMA:MMA, but were negative predictors of %MMA .

In crude analysis, dietary total As explained approximately 16% of the variance in urinary total As, 14% of the variance in sum of species, 10% in DMA:MMA, and 5% in %MMA. Dietary inorganic As had r-squared values of 7% or less for all of the urinary biomarkers. Urine creatinine was associated with all of the urinary biomarkers, and alone explained 29% of the variance in total As, 31% in sum of species, 15% in DMA:MMA, and 3% in %MMA. There was, however, no relation between dietary As and creatinine (see Appendix J).

In the crude models, females had significantly lower urinary total and sum of species As, compared to males, but sex showed no effect on other urine biomarkers. Age was negatively associated with sum of species As, and BMI was negatively associated with %MMA and positively associated with DMA:MMA. Current smoking status was a positive predictor of %MMA and a negative predictor of DMA:MMA.

Table 8.15. Crude predictors of urinary arsenic biomarkers, NHANES 2003-04.

	Total As[1] (µg/L)	Sum of species As[1] (µg/L)	% MMA	DMA:MMA
Dietary Total As[1]				
β(SE)	0.537(0.042)	0.238(0.022)	-0.036(0.003)	0.246(0.022)
P-value	<0.001	<0.001	<0.001	<0.001
Dietary Inorganic As[1]				
β(SE)	0.353(0.054)	0.238(0.031)	-0.024(0.007)	0.193(0.042)
P-value	<0.001	<0.001	0.002	<0.001
Seafood (yes/no)				
β(SE)	0.521(0.051)	0.186(0.033)	-0.035(0.004)	0.217(0.026)
P-value	<0.001	<0.001	<0.001	<0.001
Creatinine[1]				
β(SE)	0.855(0.050)	0.415(0.017)	-0.032(0.006)	0.350(0.023)
P-value	<0.001	<0.001	<0.001	<0.001
Female				
β(SE)	-0.110(0.036)	-0.060(0.018)	-0.003(0.004)	-0.017(0.021)
P-value	0.008	0.005	0.467	0.451

Table 8.15 (continued)

	Total As[1] (µg/L)	Sum of species As[1] (µg/L)	% MMA	DMA:MMA
Age				
β(SE)	-0.000(0.001)	-0.001(0.000)	-0.000(0.000)	-0.000(0.000)
P-value	0.809	0.015	0.130	0.868
BMI				
β(SE)	0.003(0.002)	0.000(0.001)	-0.001(0.000)	0.003(0.001)
P-value	0.069	0.917	0.007	0.030
Current smoking				
β(SE)	-0.055(0.039)	-0.014(0.016)	0.017(0.004)	-0.074(0.017)
P-value	0.180	0.420	<0.001	0.001

[1] values log(10)-transformed

Adjusted models

The multivariable regression results for the effects of dietary total As on urinary As biomarkers are presented in Table 8.16, and the effects of dietary inorganic As in Table 8.17. Sex, age, ethnicity, BMI, and current smoking were included as covariates in all of these models. Creatinine was not a variable of interest, nor was it a confounder of the relationship between dietary and urinary As. As previously mentioned, for purposes of comparison, the multivariable models using creatinine-adjusted urinary As values and models that include creatinine as a covariate are presented in Appendix K and Appendix L, respectively.

As in the univariate results, dietary total As was a positive predictor of urinary total, sum of species As and DMA:MMA, and a negative predictor of %MMA, after adjustment for covariates (all *P*-values <0.001) (Table 8.16). Urinary total As, sum of species and DMA:MMA tended to be higher among Mexican Americans, non-Hispanic blacks and other races as compared with non-Hispanic whites in the U.S. Percent MMA was not statistically significantly lower in Mexican-Americans as compared with non-Hispanic whites, but was significantly lower in blacks, other Hispanics and other races. Sex, age and BMI had no effect on urinary

biomarkers, but current smoking was associated with higher %MMA and lower DMA:MMA (both *P*=0.004). Most of the models with dietary inorganic arsenic showed similar trends to those with dietary total arsenic (Table 8.17), but there was no relation between dietary inorganic As and %MMA. The best fitting model with dietary inorganic As was the urinary sum of species model, which had an adjusted R^2 of 0.134.

Restricted analysis: Non-seafood eaters

Although seafood was consumed by only 12% of the NHANES population, seafood consumption the previous day was positively associated with both dietary total As and urinary total As in crude models. Any reported seafood consumption accounted for approximately 46% of the variance in dietary total As and 13% of the variance in urinary total As (not shown).

In multivariable models that were restricted to non-seafood eaters, the associations between dietary total As intake and urinary total, sum of species and DMA:MMA were similar to those observed in unrestricted models, but there was no association between dietary total As and %MMA in the restricted model(not shown).

Table 8.16. Multiple linear regression models of effect of log(10) adjusted dietary total arsenic on log(10) urinary As biomarkers, NHANES 2003-04.

	Urinary Total As	Urinary Sum of Species As	Urinary %MMA	Urinary DMA:MMA
Dietary total As				
β(SE)	0.500(0.047)	0.206(0.022)	-0.029(0.003)	0.211(0.020)
P-value	<0.001	<0.001	<0.001	<0.001
Sex (female)				
β(SE)	0.075(0.040)	-0.043(0.023)	-0.005(0.006)	0.002(0.027)
P-value	0.082	0.083	0.404	0.940
Age				
β(SE)	0.001(0.001)	-0.000(0.000)	-0.000(0.000)	0.000(0.000)
P-value	0.538	0.284	0.191	0.486
BMI				
β(SE)	-0.000(0.002)	-0.001(0.001)	-0.001(0.000)	0.002(0.002)
P-value	0.887	0.479	0.188	0.408
Current smoking				
β(SE)	-0.056(0.032)	-0.021(0.016)	0.014(0.004)	-0.065(0.019)
P-value	0.100	0.221	0.004	0.004

Table 8.16 (continued)

	Urinary Total As	Urinary Sum of Species As	Urinary %MMA	Urinary DMA:MMA
Non-Hispanic white	Ref	Ref	Ref	Ref
Mexican-American				
β(SE)	0.115(0.029)	0.112(0.020)	-0.008(0.006)	0.076(0.024)
P-value	0.001	<0.001	0.232	0.007
Other Hispanic				
β(SE)	0.166(0.115)	0.169(0.074)	-0.029(0.013)	0.196(0.097)
P-value	0.170	0.037	0.036	0.062
Non-Hispanic black				
β(SE)	0.194(0.054)	0.054(0.019)	-0.018(0.007)	0.110(0.035)
P-value	0.003	0.014	0.018	0.006
Other race				
β(SE)	0.241(0.083)	0.146(0.039)	-0.026(0.008)	0.155(0.043)
P-value	0.011	0.002	0.005	0.003
R^2	0.1999	0.2012	0.1068	0.1523
Adjusted R^2	0.1951	0.1964	0.1014	0.1472

Table 8.17. Multiple linear regression models of effect of log(10) adjusted dietary inorganic arsenic on log(10) urinary As biomarkers, NHANES 2003-04.

	Urinary Total As	Urinary Sum of Species As	Urinary %MMA	Urinary DMA:MMA
Dietary inorganic As				
β(SE)	0.210(0.078)	0.157(0.047)	-0.012(0.010)	0.120(0.060)
P-value	0.016	0.005	0.225	0.063
Sex (female)				
β(SE)	-0.112(0.050)	-0.049(0.027)	-0.003(0.006)	-0.009(0.033)
P-value	0.041	0.086	0.667	0.782
Age				
β(SE)	-0.00(0.001)	-0.001(0.001)	-0.000(0.000)	0.000(0.000)
P-value	0.842	0.274	0.370	0.871
BMI				
β(SE)	0.002(0.002)	-0.000(0.001)	-0.001(0.000)	0.002(0.002)
P-value	0.497	0.723	0.143	0.284
Current smoking				
β(SE)	-0.070(0.037)	-0.027(0.018)	0.015(0.004)	-0.070(0.020)
P-value	0.080	0.154	0.003	0.003

Table 8.17 (continued)

	Urinary Total As	Urinary Sum of Species As	Urinary %MMA	Urinary DMA:MMA
Non-Hispanic white	Ref	Ref	Ref	Ref
Mexican-American				
β(SE)	0.118(0.037)	0.112(0.023)	-0.008(0.006)	0.076(0.022)
P-value	0.006	<0.001	0.176	0.004
Other Hispanic				
β(SE)	0.267(0.090)	0.192(0.061)	-0.035(0.011)	0.231(0.083)
P-value	0.009	0.007	0.006	0.014
Non-Hispanic black				
β(SE)	0.243(0.065)	0.075(0.024)	-0.021(0.007)	0.131(0.040)
P-value	0.002	0.007	0.013	0.005
Other race				
β(SE)	0.379(0.121)	0.180(0.053)	-0.034(0.008)	0.203(0.045)
P-value	0.007	0.004	0.001	<0.001
R^2	0.0875	0.1339	0.0727	0.0949
Adjusted R^2	0.0820	0.1287	0.0671	0.0894

Nutrient models

Day one of the NHANES 2003-2004 dietary interview data was evaluated for nutrient intake, including total dietary folate, Vitamin B-6, Vitamin B-12 and protein. Nutrient intake was adjusted for implausible caloric reporting and log(10)-transformed prior to modeling.

In crude analyses, dietary total folate, Vitamin B-6, Vitamin B-12 and protein were positively associated with urinary total As (all *P*-values <0.015). Total folate and Vitamin B-6 were also positive predictors of urinary sum of species As (*P*=0.012 and *P*=0.024, respectively) (not shown). None of the nutrients tested were associated with either %MMA or DMA:MMA. When all four nutrients were entered into multivariable models that included dietary total As, ethnicity, age, sex, BMI, and current smoking, total protein and Vitamin B-12 remained statistically significant, that is, dietary total protein was negatively associated and Vitamin B-12 was positively associated with urinary total As. In modeling the effect of these nutrients on sum of species As, total protein showed a significant negative relationship. Overall, dietary nutrient intake explained an additional 1% of the total variance in sum of species (Table 8.18).

Table 8.18. Nutrient models: Effect of dietary total As and dietary nutrients on urinary total As and sum of species As in NHANES 2003-04.

	Urinary Total As*		Urinary Sum of Species As*	
	β(SE)	*P*	β(SE)	*P*
Dietary protein*	-0.386(0.129)	0.009	-0.216(0.070)	0.008
Dietary folate*	0.047(0.085)	0.586	0.030(0.051)	0.568
Dietary Vit B-12*	0.153(0.063)	0.029	0.027(0.037)	0.476
Dietary Vit B-6*	0.037(0.065)	0.574	0.011(0.033)	0.751
Dietary total As*	0.511(0.045)	0.001	0.221(0.020)	0.001
Sex (female)	-0.095(0.045)	0.053	-0.063(0.027)	0.036
Age	0.000(0.001)	0.979	-0.001(0.001)	0.139
BMI	0.001(0.003)	0.650	0.000(0.001)	0.671
Non-Hispanic White	ref		ref	
Mexican-American	0.119(0.027)	0.001	0.111(0.019)	0.001
Other Hispanic	0.181(0.116)	0.140	0.163(0.070)	0.035
Non-Hispanic Black	0.204(0.055)	0.002	0.052(0.019)	0.017
Other race	0.244(0.080)	0.008	0.141(0.037)	0.002
Current smoking	-0.058(0.032)	0.095	-0.021(0.018)	0.258
R^2	0.2108		0.2136	
Adjusted R^2	0.2039		0.2068	

* Log(10)-transformed values

4. Discussion

In a nationally representative population sample comprising 2420 individuals who participated in NHANES 2003-2004, dietary total and inorganic arsenic were significant predictors of As metabolites measured in urine collected the morning after the 24-hour dietary recall interview was conducted. Total protein intake from foods and beverages was independently associated with decreased sum of species As, suggesting a reduction of total absorbed As dose with increased dietary protein.

Dietary total As was positively related to total As, sum of species As and the ratio of DMA to MMA in urine, and negatively associated with percent MMA. The latter result suggests not only that there is a relationship between dietary intake and urinary excretion, but also that increased dietary As may be related to increased primary methylation efficiency. The positive relationship between dietary As intake and DMA:MMA suggests increased efficiency of secondary As methylation, as well. This association between increased methylation efficiency and increased dietary exposure is difficult to explain. Dietary inorganic As intake and urinary biomarkers showed the same

relations as dietary total As in univariate analysis, though the relation with %MMA and MMA:DMA were no longer statistically significant after adjustment.

Mean daily intake of dietary As in the U.S. population based on dietary interviews and published arsenic residue data (Schoof et al., 1999) was 41 µg/day of total As, and 6.2 µg/day of inorganic As. Dietary As varied by ethnic group and was especially high among other Hispanics (70 µg/day of total and 9.9 µg/day of inorganic) and among other races (80 and 10.3 µg/day, respectively). Blacks and Mexican-Americans also ingested higher amounts of dietary As than whites in the U.S. Dietary differences among ethnic groups have not yet been assessed, but patterns of food consumption would be expected to vary.

There were significant differences in urine creatinine levels and in As metabolites by ethnicity, but creatinine-adjustment only slightly attenuated the differences between non-Hispanic blacks (who had the highest creatinine levels) and non-Hispanic whites and Mexican-Americans and whites (who had the second highest creatinine levels). The effects of sex, age, BMI and smoking on urinary As biomarkers were inconsistent in the various models depending on whether unadjusted urinary outcomes or

creatinine-adjusted outcomes were regressed, or whether creatinine was included as a covariate. Some of the models in which creatinine was included as a covariate fit a quadratic model (with a creatinine-squared term in the model as well) better than a linear model. Also, there was interaction between creatinine and sex and creatinine and current smoking status in some of the models. There were several reasons that adjustment for urine creatinine was not presented in the primary results of this aim. First, creatinine concentration was not related to dietary As in this population, but is directly related to urinary As methylation. It uses the 1-carbon metabolic pathway and may obscure the relation between folate intake and arsenic metabolism (Gamble et al., 2005). Also, as reported in the literature, creatinine is associated with sex, age, ethnicity, BMI, and smoking (Barr et al., 2005).

Notably, the NHANES survey included no data on personal exposure to arsenic in water used for drinking or cooking, and this was a significant limitation in that it was not possible to estimate total As exposure through ingestion for the U.S. population as a whole. Despite this limitation and the potential for confounding due to the effects of unknown exposure from drinking and cooking

water, dietary As exposure alone was highly associated with urinary As biomarkers.

IX. Overall Discussion

This dissertation research focused on evaluating the effects of dietary arsenic and nutrient intake on total and speciated urinary arsenic biomarkers. Aim 1 assessed the validity of modeling dietary As intake by comparing estimated intake based on diet diaries and residue databases to measured intake from duplicate diet samples in the NHEXAS-Arizona and Arizona Border Survey populations. In these populations, measured arsenic and several modeled estimates of dietary arsenic intake were evaluated as independent predictors of urinary total arsenic excretion, with and without adjustment for subject-specific confounders including As intake from drinking and cooking water. Effects of exposure to tap water As concentrations above and below the EPA maximum contaminant level (MCL) were also assessed. Aim 2 used validated modeled estimates of dietary As and modeled estimates of drinking and cooking water As exposure to predict urinary total and methylated arsenic biomarkers in another local/regional study population--the Arizona subpopulation of the Binational Arsenic Exposure Survey (BAsES). The effects of ingested As, adjusted for age, sex, ethnicity, BMI, and smoking on

urinary total As, As sum of species (total absorbed As dose), % MMA and DMA:MMA (indicators of arsenic methylation efficiency), the relation of specific nutrients to As methylation efficiency, and the effects of tap water As concentrations above vs. below the MCL were evaluated in this aim. In Aim 3, the models developed in Aims 1 and 2 were applied to a nationally-representative population sample (NHANES 2003-2004) to determine whether the relation between dietary As and nutrient intake and urinary biomarkers observed in the regional models could be generalized to the U.S. population at large, despite the missing information on exposure to arsenic from drinking and cooking water.

Modeled vs. measured dietary As

As hypothesized, modeled dietary total arsenic intake provides a correlative approach to estimating exposure that can be used to predict urinary total As exposure. In Aim 1, modeling dietary As using Total Diet Study (TDS) mean arsenic residues resulted in a gross underestimate of intake, while modeling based on TDS maximum residues or on mean As residue data from Schoof et al. (1999) yielded much

better approximations to the measured arsenic in the duplicate diet samples.

The fact that TDS maxima and Schoof residue data, constructed on foods comprising over 90% of inorganic arsenic in the U.S. diet, is not surprising. The limit of detection for total As in TDS was more than ten times higher than in NHEXAS/ABS and Schoof, and the calculation used to obtain TDS means averaged in zeroes for food values below the LOD.

Two other studies using different modeling methods compared measured and modeled dietary As exposure in the NHEXAS-AZ population, and both found comparability between modeled and measured values (Moschandreas et al., 2002; Xue et al., 2010).

Using dietary arsenic exposure estimates based on a national residue database--Combined National Residue Database (CNRD)--yielded lower estimates than those obtained from duplicate diet residues in NHEXAS-AZ, and the authors postulate that higher dietary As residues in NHEXAS-AZ may be due to the impact of mining. However, as discussed above, we also found that using TDS mean residues--another national residue database—resulted in an underestimate of exposure. The evaluation of As exposure

in mining and non-mining communities by O'Rourke et al. (1999b), using NHEXAS-AZ data, did not find a difference in dietary As *per se*, but did find increased exposure from water and possibly from water added to beverages and foods during preparation. Moschandreas et al. (1999) also compared duplicate diet residues to estimates of mean dietary As exposure derived from NHEXAS-AZ dietary questionnaires and Dietary Exposure Potential Modeling (DEPM) based on food items ranked by contribution to selected chemical residues. The DEPM estimates were higher than the measured As, and the authors suggested that this might be due to regional differences in exposure and/or demographic differences in consumption patterns (Moschandreas et al., 2002). These DEPM results are probably more comparable to the results we obtained using the residue data from Schoof et al. (1999), which also only included "high impact" foods.

Xue and colleagues (2010) applied a Stochastic Human Exposure and Dose Simulation (SHEDS)-Dietary model to NHEXAS-AZ and reported compatibility between predicted exposure and exposure measured in duplicate diet samples. The SHEDS-Dietary estimates were slightly higher than the duplicate diet estimates for NHEXAS.

Modeling of urinary As biomarkers

Differential LODs were also an issue in modeling urinary total As. The high proportion of subjects with urinary As below the LOD in NHEXAS and ABS (36 and 57%, respectively) limited discernment of a linear effect of diet in these populations. In BAsES and NHANES, both of which had less than 1.1% of subjects with urinary As concentrations below the LOD, this was not a problem. Regardless of limitations of dietary As and urinary As data, in subjects with measurable urinary As, both measured and modeled dietary arsenic—even estimates based on TDS mean values--were predictive of urinary As outcomes.

While the impact of drinking water As on cancer and chronic health risks are well-documented, the effect on urinary As metabolism is less clear. In a study in northern Chile, a difference of 500 µg/L of As in drinking water resulted in a mean difference in percent urinary inorganic As (inorganic As/sum of species As) of only 3.5% (Hopenhayn-Rich et al., 1996b). In both this and second study that used a subset of the same population (Hopenhayn-Rich et al., 1996a), the authors reported that ingestion of inorganic As from water only played a minor role in urinary As methylation patterns. Factors such as ethnicity, sex,

cigarette smoking, duration of exposure, and probably genetics had a greater impact on the ratio of MMA:DMA (Hopenhayn-Rich et al., 1996a).

Findings reported in this dissertation show that at the exposure levels experienced in the populations described here dietary As has a greater impact on urinary total and sum of species As than arsenic in drinking water, but the relation of ingested As to methylation patterns is much less clear. In the BAsES population, dietary total As, drinking water and cooking water As were positively associated with urinary sum of species As, but not with methylation. Other factors, such as sex and BMI, explained the bulk of the variance in %MMA and DMA:MMA in BAsES, and there are other studies that have reported an effect of BMI and gender on arsenic metabolism in a Central European population (Lindberg et al., 2007) and in Mexico (Gomez-Rubio et al., 2011). The NHANES population, without adjustment for water As, showed an association between dietary total As and %MMA and DMA:MMA. Similar to the Chilean data, smoking and ethnicity were significant predictors in these models (Hopenhayn-Rich et al., 1996b).

Stratification by tap water As concentration

A secondary objective of this research was to assess the effects of dietary arsenic in subjects exposed to water As concentrations above versus below the current EPA standard of 10 ppb, to elucidate the potential impact and efficacy of the current EPA regulations. In subjects exposed to tap water arsenic below the MCL, differences in dietary As intake were expected to account for most of the variability in urinary arsenic. However, in NHEXAS, ABS, and BAsES, dietary As was a significant predictor of urinary total and sum of species As regardless of stratification by tap water As. Arsenic in drinking water was not a predictor of urinary As in subjects living in households with water As below the MCL. The stratified analyses underscore the importance of dietary As to total As exposure in U.S. populations.

These results were corroborated in a study in Bangladesh, in which dietary As exposure was stratified by groundwater As concentrations (> 50 µg/L, > 10 µg/L, and < 10 µg/L). In subjects exposed to well water with As < 50 µg/L, dietary As was the predominant source of exposure (Kile et al., 2007b). In a two-month intervention that provided water with < 45 µg/L arsenic to a population in northern Chile

that had previously been chronically exposed to concentrations > 600 µg/L, there was a significant decrease in mean urinary sum of species As after the intervention, but only a slight decrease in percent urinary inorganic arsenic (inorganic/sum of species As) and MMA/DMA ratio (Hopenhayn-Rich et al., 1996a).

Ethnic differences

A few studies have reported ethnic differences in the proportion of As metabolites in populations exposed to elevated As in drinking water (Tseng, 2009). Various indigenous population subgroups in the Andes of northern Argentina and in the Yaqui Valley of Sonora, Mexico appear to have much lower proportions of MMA in the urine as compared with most other populations (Concha et al., 1998; Meza et al., 2004). Differences in arsenic methylation capacity among ethnic groups might be partly attributable to genetics, but diet, nutritional status, duration of exposure and other factors are all potential confounders (Tseng, 2009). Consumption of seafood, in particular, has been shown to directly increase excretion of DMA in some studies (Lovreglio et al., 2012).

Culture, geography, age, lifestyle and other factors influence dietary behaviors around the world and within the U.S., and various ethnic or cultural groups may be differentially exposed to dietary contaminants, such as arsenic (Cleland et al., 2009). Certain foods high in arsenic, such as rice, chicken, shellfish, finfish, seaweed, and apple juice, may be consumed more frequently or in greater quantities by subgroups of the population.

Due to presumed cultural dietary differences and also possible genetic differences in the metabolism of arsenic, Hispanics from Arizona and Hispanic-Americans and possibly Asian-Americans in NHANES were expected to have higher levels of dietary As intake, higher concentrations of As in urine, and potentially different methylation patterns than non-Hispanics in these populations. Instead, differences by ethnic group were somewhat inconsistent across study populations. In NHEXAS/ABS, Hispanics had higher mean dietary As exposure than non-Hispanics and lower mean exposure from drinking and cooking water, but there was no difference in mean concentration of total As in the urine. In contrast, mean arsenic exposure from food and water in Hispanics and non-Hispanics in BAsES showed no differences, but mean urinary total As and MMA were significantly lower

among Hispanics. In NHANES, mean dietary total As was significantly higher in "other races," "other Hispanics," and non-Hispanic blacks as compared with non-Hispanic whites. Dietary inorganic As was significantly higher in "other races," "other Hispanics," and Mexican-Americans (listed in order from highest to lowest).

Our research found no evidence that Hispanics were exposed to higher concentrations of arsenic in drinking or cooking water than non-Hispanics. Although the BAsES study showed no ethnic differences in dietary exposure, Hispanic whites and other minorities in NHEXAS, ABS and NHANES consumed higher levels of arsenic in their diets, as compare to non-Hispanic whites. Whether these differences are attributable to dietary differences due to culture or socioeconomics and/or to consumption of certain foods high in arsenic needs to be further explored.

Understanding the effects of ethnicity on urinary As in NHANES is complicated due to confounding by urine creatinine. Creatinine levels are significantly different between ethnic groups, and are especially high in non-Hispanic blacks. Without adjustment for creatinine, urinary total As was significantly lower (μg/L) among non-Hispanic whites as compared with all other ethnic groups.

After adjustment, urinary As, total and speciated (µg/g creatinine) in non-Hispanic whites was significantly lower than in "other races" and "other Hispanics," but not significantly different from the other ethnic groups. There were no differences in total or sum of species As in Mexican-Americans as compared with non-Hispanic whites in NHANES, but Mexican-Americans had a significantly higher mean DMA concentration as compared with non-Hispanic whites. In contrast, DMA was lower in Hispanics than in non-Hispanics in the BAsES study (borderline significant).

Nutrient intake and urinary As

The hypothesis that increased dietary intake of folate, protein, methionine, Vitamin B-12, Vitamin B-6 would result in increased As methylation was tested in Aims 2 and 3 of this dissertation. Models of the relation between dietary nutrient intake and urinary As biomarkers showed statistically significant effects. In adjusted models in both BAsES and NHANES, increased protein intake was associated with decreased urinary total As and absorbed dose. In crude analyses in the BAsES population, increased dietary protein was also associated with deceased % MMA and increased DMA:MMA.

Dietary habits and nutrition have received scant attention either for their potential role in modulating the health effects of contaminant exposure or as potential explanatory factors for the variability in assessment of risk from these exposures (Hennig B, 2012). In the case of arsenic, toxicity is thought to be directly or indirectly related to nutritional factors. Animal and epidemiological studies have shown some evidence that nutritional intervention could potentially mitigate arsenic-induced health effects, including cancer (Anetor et al., 2007) and skin lesions (Pierce et al., 2011). Many nutrients have been hypothesized to play a role in arsenic metabolism, but the results of various studies have been inconsistent.

Previous research on dietary nutrients and As metabolism has largely been conducted in undernourished populations, in Bangladesh, India, and Taiwan, and it is unclear whether these populations, with generally low intake of protein, have sufficient levels of methionine, choline and/or cysteine to metabolize inorganic As (Steinmaus et al., 2005b). Malnourishment, in general, has been associated with the development of arsenic-induced skin lesions (Guha Mazumder et al., 1998) and skin cancer (Fawell, 1995). Heck et al. (2007) found that higher intake of cysteine,

methionine, protein, calcium, and vitamin B-12 were associated with higher ratios of urinary MMA to inorganic As (increased primary methylation) in a study of over 1000 Bangladeshi adults (Heck et al., 2007). Another study by Heck et al. (2009) looked at dietary intake of protein, methionine and cysteine in over 10,000 (disease-free) inhabitants of Bangladesh who were participants in a longitudinal study of arsenic exposure. Using food frequency questionnaires to ascertain nutrient intake, greater dietary intake of these nutrients were associated with increased urinary total arsenic excretion, after adjustment for water arsenic intake, age, sex, body weight, tobacco use, total energy intake, and intake of certain other nutrients (Heck et al., 2009). These findings are contrary to our findings in BAsES and NHANES, in which protein intake is inversely related to total and sum of species arsenic in adjusted models. The study by Heck and colleagues did not include dietary As intake in their models and NHANES did not include water As, but the other confounders in both studies were very similar. Nevertheless, more than a third of the Bangladeshi study population had extremely low BMI (<18.5), and the overall

population was younger and undoubtedly differed in exposure to other potential risk factors (Heck et al., 2009).

A study in the western U.S. of 87 subjects living in areas with historically high water As levels looked at the relation between dietary nutrient intake and arsenic methylation for 30 nutrients. Nutrient intake was adjusted for total caloric intake, and age, sex, current smoking, and urinary sum of species arsenic were included as covariates in the regression models. Each model included only a single nutrient. In both crude and adjusted models, subjects in the lowest quartile of protein intake, as compared with the upper quartile, excreted a higher proportion of inorganic arsenic as MMA (Steinmaus et al., 2005b). In separate models, they found no relationship between folate intake and any measures of arsenic metabolism. These results were consistent with the nutrient results in the BASES and NHANES studies.

Comparison of dietary and urinary As in study populations

A comparison of dietary total and inorganic As exposure estimates based on Schoof et al. (1999) and urinary total As in NHEXAS-AZ, ABS, BAsES and NHANES are shown in Appendix M. Mean dietary total As intake in these studies

ranged from 32.8 µg/day in BAsES to 85.5 µg/day in ABS, which are in the same range as reported for U.S. adults based on the 1991-1997 FDA Total Diet Study and the 1987-1988 Nationwide Food Consumption Survey (Tao and Bolger 1999).

Urinary total As was approximately three times higher in the BAsES study than in NHANES, NHEXAS-AZ or ABS (medians of 22.6 µg/L as compared to 8.2, 10.0 and 2.35 µg/L, respectively). This difference is largely attributable to intentional selection of sampling locations in BAsES representing exposure to relatively high and low water arsenic. Although the median sum of species As was higher in BAsES than in NHANES (10.0 vs. 6.1 µg/L), percent MMA (MMA/sum of species As) was lower (12.0% and 13.6%, respectively) (Appendix N). These values are comparable to those reported for Sonora, Mexico (Meza et al., 2004), southern Arizona (Burgess et al., 2007), and in the U.S. population (Caldwell et al., 2009).

Arsenic exposure in µg/kg/day

A provisional tolerable daily intake (PTDI) recommendation for ingested (inorganic) As of 2.1 µg/kg body weight (BW) was proposed by the twenty-seventh Joint

FAO/WHO Expert Committee on Food Additives (FAO/WHO, 1983). For comparison with the PTDI recommendation and to examine exposures across studies, total and inorganic arsenic exposure was computed as µg As/kg of body weight (BW)/day.

Table 9.1 shows total and inorganic As exposure estimates in µg/kg BW/day for subjects in our study populations who did not report consumption of seafood during the previous 24 hours. Seafood eaters were excluded to remove the effect of seafood consumption on total ingested arsenic, based on the assumption that most of the arsenic in seafood is non-toxic. Total As exposure was calculated as the sum of: 1)dietary total As (derived from dietary recall data and Schoof mean As residue data (Schoof et al., 1999), 2)As ingested from drinking water, and 3)As from water used in food preparation, divided by each subject's body weight in kilograms. Inorganic As exposure was estimated using inorganic As residue data from Schoof et al. (1999). Because there was no water As data available for NHANES, exposure estimates were based solely on dietary intake.

Table 9.1. Estimated arsenic exposure (µg/kg BW/day) based on dietary[1] and water[2] As intake in non-seafood eaters in the four study populations.

	NHEXAS-AZ	ABS	BAsES	2003-04 NHANES[3]
Total As (µg/kg/day)				
Median	1.081	1.069	0.471	0.472
90th percentile	2.067	2.531	1.462	1.130
95th percentile	2.582	4.126	2.246	1.491
> 2.1 µg/kg/day	8.9%	15.3%	4.6%	2.7%
Inorganic As (µg/kg/day)				
Median	0.251	0.177	0.202	0.086
90th percentile	0.644	0.604	0.810	0.219
95th percentile	0.874	0.805	1.453	0.312

[1]Based on Schoof et al., 1999; [2]drinking and cooking water; [3]No water As data available for NHANES.

The median total arsenic exposure among non-seafood eaters was 1.1 µg/kg/day in both NHEXAS-AZ and ABS, and approximately half that (0.47 µg/kg/day) in BAsES and NHANES. Greater than 15% of subjects living along the Arizona-Mexico border ingested >2.1 µg/kg/day, the PTDI, and 8.9% of NHEXAS, 4.6% of BAsES, and 2.7% of NHANES exceeded the PTDI.

Various estimates of dietary exposure to arsenic in U.S. populations have been published. Gunderson et al. (1995) reported mean daily intake of As by age group, based on the U.S. FDA Total Diet Study from 1986-1991. Adult males and females ingested an average of 0.44-0.51 µg/kg BW/day. These values are comparable to the median exposure estimates for BAsES and NHANES, which include both food and water. Using SHEDS-dietary modeling, Xue and colleagues (2010) estimated mean total and inorganic As exposure from food of 0.38 µg/kg/day and 0.05 µg/kg/day, respectively, and mean inorganic As exposure from water of only 0.025 µg/kg/day (Xue et al., 2010). Moschandreas et al. (2002) used arsenic residue data from the NHEXAS-AZ duplicate diet samples and body weight from questionnaires to obtain an estimate of mean exposure to total As from food of 0.70 µg/kg/day (Moschandreas et al., 2002), closer to our

estimates for NHEXAS and ABS, which included water as well as food.

Limitations

Bias is inherent in dietary assessment and both random and systematic measurement errors occur (Ferrari et al., 2008). In an analysis of 37 studies, 30% of subjects under-reported energy intake by approximately 15%, regardless of the method of dietary assessment (Poslusna et al., 2009). However, subject characteristics such as BMI, age, sex, socioeconomic status, psychological factors, literacy, language, culture, respondent training and eating habits can also influence the accuracy of dietary data and misreporting (Poslusna et al., 2009; Thomas et al., 1997). In the present study, we evaluated the relationship of BMI, age, sex, ethnicity and study population on reported energy intake, and attempted to control for physiologically implausible caloric intake (Huang et al., 2005) using standard equations (Mifflin et al., 1990) and adjusting caloric intake only in those subjects who appeared to grossly over or underestimate intake.

A major impediment to modeling dietary As is the dearth of data on food residues. Recent studies have documented

extensive variability in total and speciated As content of foods, both within and between brands (Cascio et al., 2011; Roberge et al., 2009). It is clear that heterogeneity of source due to local conditions—including past use of arsenical pesticides, arsenic in irrigation water and/or water used in food processing—are possible explanations for widely divergent reported concentrations (Meharg and Raab, 2010; Xue et al., 2010). The complex matrix of food and the different arsenic compounds and valence states further complicate laboratory analysis (Meharg and Raab, 2010). As discussed above, there were major differences in the LODs and the handling of values below detection in the calculation of TDS mean As residue values, as compared with the duplicate diet samples and the Schoof data (Schoof et al., 1999), that probably account for the low estimates of dietary intake based on TDS means.

There were heterogeneities among the studies used in this dissertation, including marked differences in study design, recruitment, time periods during which the studies were conducted, laboratory methods, and differences in age and sex distribution. These surveys were all cross-sectional in nature and there was no way to assess intra-individual variation in dietary or other exposures. Although it was

assumed that any misclassification that might result from the study design would be non-differential, this assumption was not specifically tested and probably does not hold for BAsES.

In particular, in BAsES, study sites were selected for recruitment on the basis of groundwater As concentration and not selected at random, and in the analysis of the data, mixed model regression was used to control for potential clustering within communities and households. However, despite random recruitment of households within the selected communities and the use of mixed models, there could be residual confounding related to unknown or unmeasured exposures.

In the 2003-2004 NHANES study, there was no collection or analysis of water samples for arsenic, nor was specific geographic information available for the participants. Hence, it was not possible to evaluate arsenic exposure from ingestion of water or to adjust the models for subject-specific, regional or ethnic differences in water As intake.

Potential modeling issues related to differences in the minimum detection limit for urinary As in the four populations were discussed above. Urine As concentration

depends on hydration and the amount of time since last urination, and inconsistencies between study populations due differences in hydration status are possible. Both specific gravity and creatinine are commonly used to correct for urine concentration, and both methods are considered valid approaches (Miller et al., 2004), though they may not produce comparable results. Specific gravity is a ratio of the density of a urine specimen to the density of water, and creatinine, a byproduct of muscle activity, is used as a measure of kidney function. However, there is extensive inter- and intra-subject variability in urine creatinine, and factors such as ethnic or racial group, age, sex, physical activity, BMI and diet influence the concentration (Miller et al., 2004; Barr et al., 2005; Hopenhayn-Rich et al., 1996a). Hence, adjusting for creatinine can have significant effects on these covariates, and interpretation is complex when urinary metabolites are expressed in per gram creatinine (Gamble and Liu, 2005).

Other approaches include adding creatinine as a separate independent variable (Barr et al., 2005), but Gamble and Liu (2005) noted significant complications of including creatinine in studies of arsenic methylation and folate, in

that creatine, a precursor of creatinine, involves one-carbon metabolism and folate-dependent methylation. They found that creatinine was a confounder of the relationship between folate and urinary As (Gamble and Liu, 2005).

In NHEXAS and ABS studies, no correction for hydration status was used. In BAsES, urinary As measures were adjusted for specific gravity. The NHANES analysis was conducted three ways, urinary As unadjusted for creatinine, urinary As per gram creatinine, and with creatinine included as a covariate in the regression models. Interpretation of the unadjusted results was more straight-forward and these were presented as main results; the creatinine-adjusted results are in the Appendices for comparison.

In this dissertation, extensive consideration was given to addressing possible biases and potential confounding due to these and other factors measured in these studies. However, other sources and modes of environmental arsenic exposure were not measured in these populations, but could have contributed to confounding in these models in unknown ways.

X. Conclusions

Dietary arsenic, alone and in combination with arsenic ingested from drinking water and water used in food preparation, contributes significantly to total arsenic exposure. Median exposure from food alone is 3.3-8.5 times higher than the EPA maximum contaminant level for drinking water, yet there are currently few standards in the U.S. regarding arsenic in food, and these apply only to the products of animals (e.g., eggs, poultry) treated with arsenical veterinary drugs.

Modeled dietary arsenic estimates based on residue databases correlate well with measured arsenic in composite food samples but may not yield accurate estimates of intake. However, modeled estimates of dietary exposure are better predictors of urinary arsenic than estimates of exposure to arsenic from drinking and/or cooking water. This is especially true in the U.S., where water arsenic concentrations are generally low.

The importance of food and beverages to total exposure to arsenic should instigate efforts to improve monitoring of arsenic and to establish standards for arsenic in foods.

XI. Recommendations for future research

There is a need for further research to better characterize human exposure to arsenic in food. Modeled dietary arsenic intake estimates are limited by the dearth of laboratory data on arsenic species in foods and in composite food samples (as prepared). It is clear from published studies that there is a large amount of variation in the arsenic content of the relatively few foods that have been repeatedly analyzed, and in the proportion of methylated arsenic species in these foods.

Assessment of speciated arsenic—specifically MMA and DMA—in duplicate food samples, along with urine samples from a controlled study of dietary intake over a period of several days to a week, and during different harvest seasons, would greatly enhance our understanding of intra- and inter-individual variation in As methylation. Furthermore, nutrient intake could be monitored and/or modified to pinpoint any effects of specific nutrients on methylation. A comparison, then, with modeling dietary arsenic from food records and existing residue databases could elucidate the limitations of modeling.

There is also inadequate information on the arsenic residues in different types of seafood, and on the contribution of different types of seafood to urinary As species.

How food crops accumulate arsenic compounds and on whether plants are capable of methylating arsenic or are obtaining methylated arsenic species from historic pesticide use or other anthropogenic sources are not well understood. There is a critical need for additional research on arsenic uptake in different food crops, arsenic speciation in plants, and efficacy of phosphorus fertilizers in mitigating arsenic uptake by plants that accumulate arsenic.

APPENDIX A

LABORATORY METHODS AND LIMITS OF DETECTION FOR ANALYSIS OF ARSENIC IN WATER, FOOD AND URINE.

	Water		Food		Urine	
	Method	LOD	Method	LOD	Method	LOD
NHEXAS-AZ/ABS	ICP-MS	0.20 µg/L	ICP-MS	Total: 0.19-2.7 µg/kg	ICP-MS	Total: 4.1 µg/L
BAsES	ICP-MS	0.1 µg/L			ICP-MS	Speciated: 0.1-0.12 µg/L
2003-04 NHANES					ICP-MS	Speciated: 0.6-1.2 µg/L
Total Diet Study (1991-2005)			HG-AAS	Total: 40 µg/kg		
Schoof et al. (1999)			ICP-MS	Total: 3.6 µg/kg Inorganic: 2.0 µg/kg		

APPENDIX B

PROTOCOL FOR ACCESSING NHANES FILES FROM THE NATIONAL CENTER FOR HEALTH STATISTICS (NCHS)

Data Sets and Related Documentation: NHANES 2003-2004

Use SAS VIEWER to view data downloads and then save as tab-delimited ASCII files (downloaded via NCHS website)

Save Directory: EPA STAR Grant 2007-As & Diet/NHANES/
/DEMOGRAPHIC DATA 2003-04 update10-29-08
/EXAMINATION DATA 2003-04 update 2-08

Survey Operations Manuals, Brochures, and Consent Documents, including procedure manuals: (http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/current_nhanes_03_04.htm)

2003-2004 Demographics File

(http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/demo03_04.htm)

DICTIONARY --> Demographics Variable List (updated October, 2008). Renamed vardemo_c.pdf--> vardemo_c-demographic-vars-dct.pdf

DATA --> demo_c.xpt --> demo_c-demographic-data _NHANES03-04.txt [tab-delimited ASCII]

DOCUMENTATION (demo_c-documentation.pdf)

QUESTIONNAIRE (http://www.cdc.gov/nchs/data/nhanes /nhanes_03_04/sp_dmq_c.pdf [renamed]-->sp_dmq_c-demog-questionnaire.pdf)

2003-2004 Examination Files

(http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/exam03_04.htm)

Dietary Interview (Individual Foods -- First Day)

[Data, Docs, Food Codes, Modification Codes, Procedures]

DATA --> dr1iff_c.pdf [renamed]--> dr1iff_c-dietary interview-day1-documentation.pdf

DOCUMENTATION --> dr1iff_c-diet-interview-day1-indiv-foods.pdf

MODIFICATION CODES --> drxfcd_c-modification-codes.txt

Dietary Interview (Total Nutrient Intakes -- First Day)

[Data, Docs, Procedures (In Person)]

DOCUMENTATION --> http://www.cdc.gov/nchs/data/nhanes /nhanes_03_04/dr1tot_c.pdf

2003-2004 Laboratory Files (Lab 06 Urinary Total Arsenic and Speciated Arsenics)

[Data, Docs]

(http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06uas_c.pdf --> L06uas_c_c-lab6-urineArsenic-doc.pdf)

APPENDIX C:

METHODS OF ASSESSMENT AND ADJUSTMENT FOR IMPLAUSIBLE KILOCALORIE REPORTING

Compute adjustment factor for extreme over and under-reporting:

MISSING BODY WEIGHT:

For children missing body weight, we used the average weight, based on age and percentile of height from growth charts for the U.S. population; for adults missing weight, we used the means of males and females in the population.

ADULTS:

- **Used** Mifflin-St Jeor equations to calculate expected kcals for adults, then computed the percent of the Mifflin-St Jeor predicted kcals that were reported in diet diaries and flagged individuals who reported eating < 50 or greater than 150% of expected kcals. Assumed sedentary lifestyle (activity factor = 1.2).

Males: BMR = (10 × weight(kg)) + (6.25 × height(cm)) – (5 × age(yrs)) + 5

Females: BMR = (10 × weight(kg)) + (6.25×height(cm)) – (5 × age(yrs)) – 161

CHILDREN:

Energy constants and equations in James and Schofield (1990) used to predict total energy expenditure (TEE) for children by sex and age.

Children < 10 years: TEE = Energy constant * weight

Children 10-17 years: TEE = (PAL)(BMR)

Individuals who reported eating < 50 or greater than 150% of expected kcals were flagged.

COMPUTE ADJUSTMENT FACTOR FOR OUTLIERS:

- For extreme excess caloric reporting:

  ADJ FACTOR = (expected kcals + 25%)/reported kcals

- For extreme under-reporting:

  ADJ FACTOR = (expected kcals/reported kcals)

Adjust measured and modeled dietary As and nutrient factors for excessive or inadequate caloric reporting by multiplying value by the adjustment factor.

References:

James, W.P.T. & Schofield, E.C. 1990. Human energy requirements. A manual for planners and nutritionists. Oxford, UK, Oxford Medical Publications under arrangement with FAO.

Mifflin, MD, St Jeor, ST, Hill, LA, Scott, BJ, Daugherty, SA, Koh, YO. 1990. The new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 51:241-7.

FAO/WHO/UNU. 2001. Human energy requirements. Report of a a Joint FAO/WHO/UNU Expert Consultation. Rome, 17-24 October 2001. 103 pp.

APPENDIX D

COMPARISON/ASSESSMENT OF KILOCALORIE REPORTING

BY STUDY POPULATION

Mean (SD) age, BMI, number of food items recorded, kilocalories reported, and expected kilocaloric intake by study population. Geometric means (95% CI) are shown for NHANES.

	NHEXAS-AZ	ABS	BAsES	NHANES 2003-2004
Age	41.6(20.5)	48.4(15.3)	55.4(15.1)	39.1(37.6-40.6)
BMI	25.0(5.6)	27.4(5.9)	29.1(6.4)	27.0(26.5-27.5)
N food items	24.7(8.2)	15.5(5.2)	31.3(13.0)	15.4(14.9-15.9)
Reported kcals	3511(1275)	1844(699)	2042(1126)	2224(2178-2269)
Adjusted kcals[1]	2242(493)	1755(396)	1863(473)	2074(2047-2101)

The table above compares the mean caloric intake reported in the dietary records, the expected kilocalories, the number of food items recorded in the diaries or interviews, and the BMI for each study population. NHEXAS-AZ, in particular, appeared to over-report kilocalorie intake. In

fact, the average reported intake was > 200% of the expected. There was no significant correlation between caloric intake and BMI or body weight, nor was there correlation between reported kilocalories and measured dietary arsenic.

In NHEXAS,

- There was only one person who reported eating <75% of expected kilocalories and 114 adults and 15 children who reported eating > 150% of expected kcals.
- There was a significant correlation between the number of food items recorded and the number of kcals (Spearman's rho=0.683, p<0.001).
- Number of kcals in duplicate diet sample was significantly correlated with body weight (Spearman's rho=0.198, p=0.008).
- Number of kcals reported was not associated with income, though at the highest tertile of income, kcals were lower.
- There was no difference in kcal reporting by ethnicity.

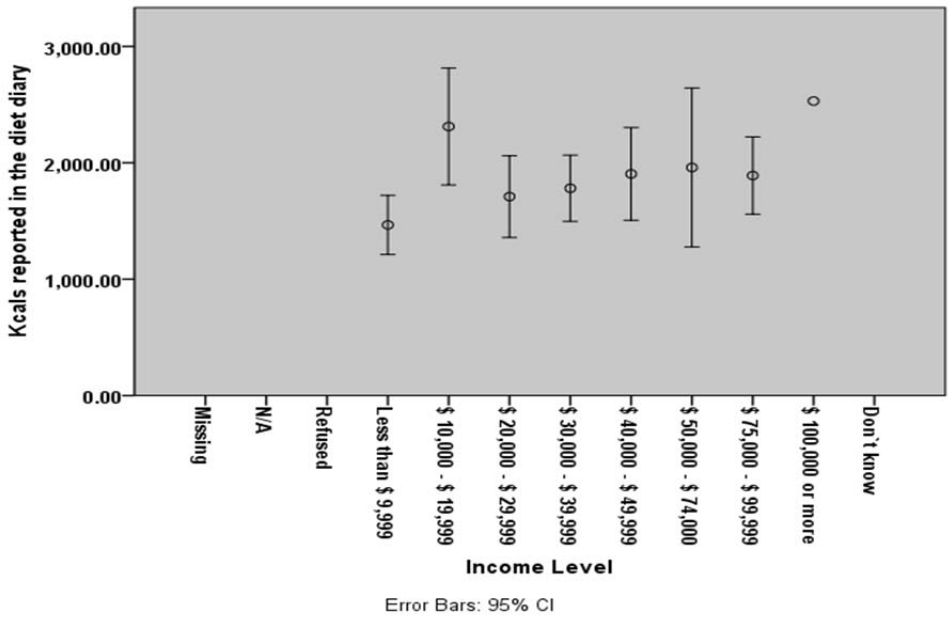
In ABS,

- % of subjects who reported extremes:

19.8% under-reported kcals

17.4% over-reported kcals

- Subjects reported an average of 3 times more grams of food consumed than they collected in the duplicate diet sample.
- Kcals are significantly correlated with the number of food items reported in the duplicate diet and in the diary.
- The mean kcals reported was markedly higher in the 18 % of the population that earned between $10,000 and $20,000 annually.



In BAsES,

- Mean kcals were higher than expected kcals, but showed a very wide range (minimum-maximum, 466-9295). Over 40% of the population reported implausible energy intake:

<75% expected kcals: 17.8%

>150% expected kcals: 24.4%

- Kcals were correlated with number of food items in diet records (rho=0.537, p.001) and with body weight (rho=0.205, p=0.002).
- There were some statistically significant differences in caloric reporting by education level, with those with the least and most education reporting the fewest calories. Subjects with some college reported significantly more kcals than high school graduates (ANOVA, Bonferroni post-hoc test p=0.019).
- There was no difference by ethnic background.

In NHANES,

- Kcals were positively correlated with body weight (r=0.117, p<0.003), height (r=0.310, p<0.001), but not with BMI.
- 17.2% reported consuming <75% expected kcals

o 21% and 27% of other Hispanics and non-Hispanic blacks, respectively under-ate or under-reported caloric intake as compared with the other ethnic groups (all 15-16%).

- 24.6% reported consuming >150% of expected kcals

o 26% of non-Hispanic whites and Mexican-Americans over-ate or over-reported caloric intake as compared with 20-23% of the other ethnic groups.

APPENDIX E

PROTOCOL FOR GENERATION OF NHEXAS-AZ AND ABS DIET DIARY DATA AND ASSIGNING ARSENIC VALUES BASED ON TDS AND SCHOOF

6/4/09, revised 11/29/09

Methods and Procedures

1. Convert NHEXAS-AZ data codes to Nutritional Data System for Research (NDSR) usable codes:
   a. "Food keys", a rapid entry format for specifying foods used by the NDSR system, will be developed for all foods in the NHEXAS-AZ database.
   b. For example, the food key for "Pear, raw" would be "PEAR.FRS", where "FRS" means "fresh".
   c. In developing a food key for a particular food, decisions often need to be made regarding how it is prepared.
      i. When it is unclear or unspecified in the NHEXAS food description, "unknown" will be used in the NDSR food key. This will automatically generate a composite of foods based on national consumption rates.
      ii. For example, "Beans, refried" would be "BEAN.REFR.U", where "U" specifies "unknown"

regarding the preparation (canned, prepared from a recipe, or a dip).

iii. NDSR will then generate a nutrient profile based on a composite of the three preparations that itself is based on NHANES national consumption rates.

2. The NHEXAS database may specify a food for which several food lines will have to be keyed out. NHANES national consumption data (1999-2002) will be used to derive the proportion of each food used in the composite.

a. For example, two food keys would be generated for "Beans, snap green, fresh/frozen, boiled": "BEAN.SNA.GRE.FRS" and "BEAN.SNA.GRE.FRZ" – one line for fresh and one for frozen. The NHANES national consumption numbers for each of these are 1085984 and 752247 respectfully.

b. Therefore, the Fresh food line as entered into NDSR will be allocated 59% of the grams [1085984/(1085984+752247)=59%], and the Frozen food line will get the remaining 41%.

3. An automated program was developed to match the data provided by "Food Keys" into NDSR software codes and

another program that matches the Food Key descriptions to the actual NHEXAS-AZ and AZ Border data. This program generates an Excel file that contains the Food Keys (representing the NHEXAS foods) that is then entered into the NDSR system. The data entry person will be able to copy and paste the exact Food Key(s) into the NDSR system. This will ensure that the same Food Key is used for a particular NHEXAS food. Other fields such as subject ID and date will also be matched.

4. Some foods do not have Food Keys (NDSR simply does not have a food key for every food). Data that cannot be auto-coded will be hand-coded and the "Key" will be a description of each step chosen, rather than a formal Food Key.

5. Quality assurance checks and final review of all data sets were performed by a nutritional database specialist.

   a. To do this we will need to generate a file that maps the Food Key to the numeric Food Code generated for the NDSR output dataset.

b. All fields (Participant ID, Date, Food Code, Grams) were compared for accuracy and a perfect match for all records.

6. Assignment of arsenic residue concentrations

a. NHEXAS and ABS used TDS codes for most of the foods recorded in the diet diaries and the duplicate diet sample.

b. Assignment of TDS total mean and total maximum arsenic values was mostly straight-forward.

i. For specific foods not listed in the TDS residue database, the As values for a closely related food item was used.

c. The same protocol as used for NHANES and BAsES food items not included in TDS or NDSR was used for NHEXAS and ABS.

APPENDIX F

PROTOCOL FOR ASSIGNING ARSENIC VALUES TO "OTHER FOODS" AND MIXED FOODS IN NHANES 2003-04 AND BAsES

Arsenic values, based on Schoof, Yost, et al., 1999.

a) Group food items to minimize the number of different foods that require recipes to determine their main ingredients.

b) Calculate the average arsenic content per food groups, based on Schoof

1. Sugars, sweets (mean of beet sugar, cane sugar and corn syrup)
2. Other fats (mean of soybean oil and butter)
3. Other dairy (mean of all dairy including butter)
4. Fish (mean of saltwater, tuna and freshwater)
5. Use mean of beef and pork for lamb or other mammals
6. Other vegetables (mean of all vegetables)
7. Other whole fruit (mean of raw apple, banana, grapes, orange, peaches, watermelon).
8. Other fruit juice (mean of apple, grape and orange juice)

9. Other grains/flours/cereals (use mean of corn meal, flour and rice)

c) Substitutions for foods not listed by Schoof:

1. Non-fish seafood: used shrimp
2. Other poultry (turkey, duck, etc.): substitute chicken
3. Soy bean products (soy yogurt, tofu, soybeans): use peas
4. Other nuts, flax seeds, sunflower seeds: use peanut butter
5. Non-dairy Creamer: use Soybean Oil
6. Whipped Topping: use soybean oil and corn syrup (primary ingredients rule)
7. Protein powders: use peas
8. Pumpkin, cantaloupe, melons, other winter squash: use watermelon
9. Cheese: average of whole milk and butter
10. Cereals and breads: use flour
11. chocolate and other candy: use sugar average
12. soy sauce: salt + water
13. Wine: use Grape juice
14. Mixed alcoholic drinks: use water and sugar

d) For mixed foods (up to 75% of grams consumed):

1. Use Food Coding Scheme that is used by FNDDS for categorizing and coding foods.
2. The NHANES Food ID number itself is part of a Food Grouping scheme.
3. The first four digits define a fairly narrow category of foods.
    a. Do a 25% sample of each "4-digit" food group (see example, below). Every fourth food would be done by the "75% of total grams" method. The rest of the foods within the "4-digit" food group would receive the average values from the sample.
        i. For example, items categorized below within 272_ code: C**ompute separate means** for chicken and rice dishes based on a 25% sample of only mixed chicken rice dishes within the 4-digit code, another 25% sample for chicken and potato dishes, another 25% sample for fish and rice, etc.
        ii. Example of Food and Nutrient Database for Dietary Studies (FNDDS) coding:

272 Meat, poultry, fish with starch item (include white potatoes)

2721 Beef with starch item (potatoes; noodles; rice; bread; Puerto Rican)

2724 Poultry with starch item (potatoes; noodles; rice; bread)

2725 Fish, shellfish with starch item

2726 Miscellaneous meats with starch item

b. Items with high arsenic in Schoof that are exceptions to the 4-digit group that they fit in (e.g., rice, fish, poultry, shrimp, grapes) are treated as exceptions.

APPENDIX G

WATER ARSENIC WEIGHTED MEAN CONCENTRATIONS

Weights were assigned to each drinking water source based on their frequency of use. Drinking and cooking water sources were reported as being consumed: frequently, moderately, rarely, and never. The frequencies were assigned a value of 3, 2, 1, and 0 respectively.

The weighted mean arsenic for each subject was calculated as follows:

$$\frac{1}{frequency_1 + frequency_2 + ... + frequency_n} \sum_{i=1}^{n} (frequency_i \times concentration\ of\ source_i)$$

n = the number of drinking sources used by the individual $frequency_i$ = the frequency of use of each source i.

APPENDIX H

CALCULATION OF ADJUSTED URINARY ARSENIC MEASURES

BAsES: Urinary arsenic values were adjusted for specific gravity[1] according to the methods of Nermell et al. (2008) (Nermell et al., 2008).

Urinary As adjusted for specific gravity =

Urinary As * (population mean specific gravity[2] – 1) / (measured SG - 1)

Units: µg/L

NHANES 2003-2004: Urinary arsenic, adjusted and unadjusted for creatinine were used in modeling. Urinary As values below the detection limit were replaced with the LOD/√2, based on an LOD of 0.6 µg/L. Urinary As values were adjusted for creatinine[3] according to the methods of (NHANES, 2007c; Caldwell et al., 2009).

Urinary As adjusted for creatinine =

(Urinary As/creatinine) * 100

Units: µg As/g creatinine

Notes:

[1] Urine specific gravity measures the concentration of particles in the urine. The normal range is from 1.003 to 1.030. Values outside of this range may be the result of specimen dilution. Elevated levels of protein in urine may cause abnormally high specific gravity values. This was used in the BAsES population to adjust for urine concentration.

[2]BAsES: Population mean specific gravity = 1.01628

[3]Urine creatinine was measured in NHANES 2003-2004. It is a waste product of creatine, an amino acid found in muscle tissue and urine. Elevated levels of protein in the urine may cause abnormally high values, and body size, nourishment, age, and gender can affect it.

APPENDIX I

CRUDE RELATION BETWEEN CREATININE-ADJUSTED URINARY TOTAL AND SUM OF SPECIES AS AND POTENTIAL PREDICTORS, NHANES

	Total As[1] (µg/g creatinine)	Sum of species As[1] (µg/g creatinine)
Dietary Total As[1]		
β(SE)	0.505(0.036)	0.209(0.026)
P-value	<0.001	<0.001
R-squared	0.195	0.081
Dietary Inorganic As[1]		
β(SE)	0.278(0.046)	0.166(0.034)
P-value	<0.001	<0.001
R-squared	0.030	0.027
Seafood (yes/no)		
β(SE)	0.552(0.037)	0.221(0.023)
P-value	<0.001	<0.001
R-squared	0.201	0.079
Female		
β(SE)	0.033(0.030)	0.083(0.016)
P-value	0.291	<0.001
Age		
β(SE)	0.003(0.001)	0.002(0.000)
P-value	<0.001	<0.001

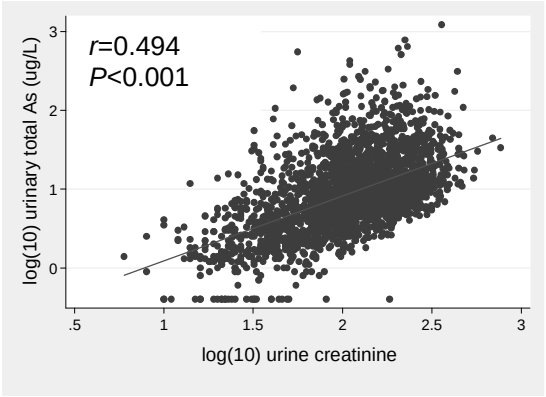
	Total As[1] (µg/g creatinine)	Sum of species As[1] (µg/g creatinine)
BMI		
β(SE)	-0.002(0.001)	-0.005(0.001)
P-value	0.152	<0.001
Current smoking		
β(SE)	-0.121(0.034)	-0.080(0.023)
P-value	0.006	0.004

[1] values log(10)-transformed

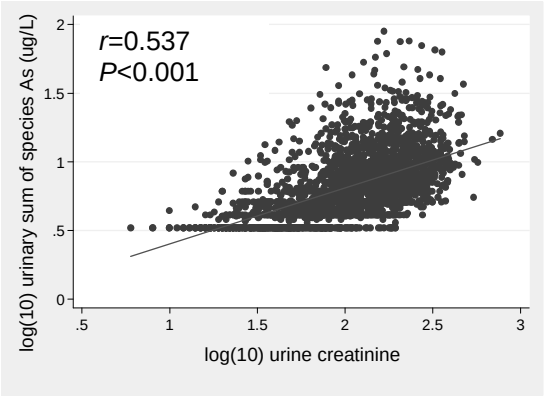
APPENDIX J

SCATTERPLOTS: RELATION OF URINARY AND DIETARY ARSENIC TO URINE CREATININE

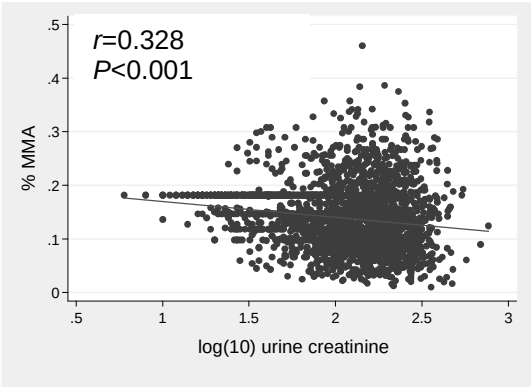
Urinary total As



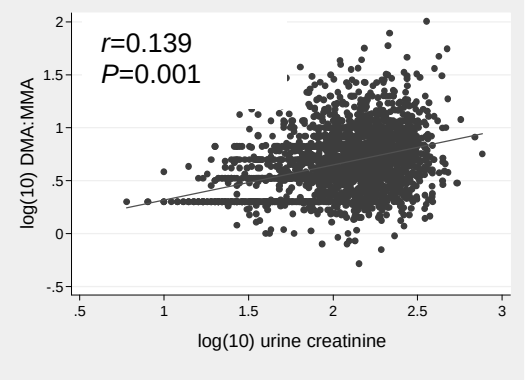
Urinary sum of species As



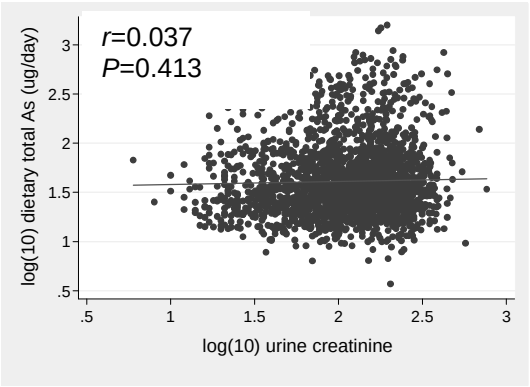
%MMA



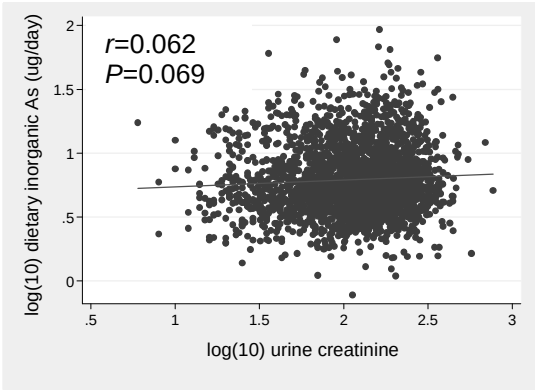
DMA:MMA



Dietary Total As



Dietary Inorganic As



APPENDIX K

MULTIPLE REGRESSION OF CREATININE-ADJUSTED URINARY ARSENIC, 2003-04 NHANES

A. LOG(10) ADJUSTED DIETARY TOTAL ARSENIC MODELS

	Urinary Total As (µg/g creatinine)*	Urinary Sum of Species As (µg/g creatinine)*
Dietary total As*		
β(SE)	0.532(0.054)	0.245(0.026)
P-value	<0.001	<0.001
Sex (female)		
β(SE)	0.097(0.034)	0.126(0.018)
P-value	0.011	<0.001
Age		
β(SE)	0.004(0.001)	0.003(0.000)
P-value	<0.001	<0.001
BMI		
β(SE)	-0.008(0.001)	-0.008(0.001)
P-value	<0.001	<0.001
Current smoking		
β(SE)	-0.075(0.032)	-0.038(0.024)
P-value	0.034	0.131

(APPENDIX K, continued)

A. (continued)

	Urinary Total As (µg/g creatinine)*	Urinary Sum of Species As (µg/g creatinine)*
Non-Hispanic white	Ref	Ref
Mexican-American		
β(SE)	0.079(0.042)	0.076(0.037)
P-value	0.081	0.058
Other Hispanic		
β(SE)	0.168(0.086)	0.169(0.040)
P-value	0.070	0.001
Non-Hispanic black		
β(SE)	0.036(0.048)	-0.103(0.024)
P-value	0.458	0.001
Other race		
β(SE)	0.235(0.059)	0.137(0.049)
P-value	0.001	0.013
Model R^2	0.2804	0.2638

* log(10) transformed values

APPENDIX K (continued)

B. LOG(10) ADJUSTED DIETARY INORGANIC ARSENIC MODELS

	Urinary Total As (µg/g creatinine)*	Urinary Sum of Species As (µg/g creatinine)*
Dietary inorganic As*		
β(SE)	0.271(0.059)	0.228(0.030)
P-value	<0.001	<0.001
Sex (female)		
β(SE)	0.064(0.040)	0.124(0.018)
P-value	0.129	<0.001
Age		
β(SE)	0.003(0.001)	0.003(0.000)
P-value	0.001	<0.001
BMI		
β(SE)	-0.006(0.001)	-0.008(0.001)
P-value	<0.001	<0.001
Current smoking		
β(SE)	-0.090(0.037)	-0.046(0.025)
P-value	0.028	0.080

(APPENDIX K, continued)

B. (continued)

	Urinary Total As (µg/g creatinine)*	Urinary Sum of Species As (µg/g creatinine)*
Non-Hispanic white	Ref	Ref
Mexican-American		
β(SE)	0.080(0.055)	0.074(0.043)
P-value	0.166	0.104
Other Hispanic		
β(SE)	0.262(0.071)	0.186(0.039)
P-value	0.002	<0.001
Non-Hispanic black		
β(SE)	0.088(0.059)	-0.078(0.026)
P-value	0.154	0.009
Other race		
β(SE)	0.365(0.080)	0.164(0.044)
P-value	<0.001	0.002
Model R^2	**0.1096**	**0.2031**

* log(10) transformed values

NOTE: Creatinine-adjustment of %MMA and DMA:MMA did not affect the coefficients in the models or the fit of the models.

APPENDIX L

MULTIPLE REGRESSION, CREATININE COVARIATE MODELS, NHANES 2003-04

A. LOG(10) ADJUSTED DIETARY TOTAL ARSENIC MODELS

	Urinary Total As*	Urinary Sum of Species As*	Urinary %MMA	Urinary DMA:MMA*
Dietary total As*				
β(SE)	0.530(0.052)	0.223(0.017)	-0.031(0.003)	0.227(0.020)
P-value	<0.001	<0.001	<0.001	<0.001
Sex (female)				
β(SE)	0.087(0.035)	0.037(0.021)	-0.012(0.005)	0.072(0.024)
P-value	0.026	0.098	0.031	0.010
Age				
β(SE)	0.004(0.001)	0.001(0.000)	-0.000(0.000)	0.002(0.000)
P-value	<0.001	0.006	0.005	<0.001
BMI				
β(SE)	-0.008(0.001)	-0.004(0.000)	-0.000(0.000)	-0.001(0.002)
P-value	<0.001	<0.001	0.533	0.454
Current smoking				
β(SE)	-0.073(0.031)	-0.029(0.017)	0.015(0.004)	-0.072(0.021)
P-value	0.033	0.096	0.005	0.003

APPENDIX L (continued)

A. (continued)

	Urinary Total As*	Urinary Sum of Species As*	Urinary %MMA	Urinary DMA:MMA*
Non-Hispanic white	Ref	Ref	Ref	Ref
Mexican-American				
β(SE)	0.081(0.041)	0.095(0.026)	-0.006(0.005)	0.061(0.019)
P-value	0.066	0.002	0.276	0.007
Other Hispanic				
β(SE)	0.167(0.087)	0.171(0.054)	-0.029(0.011)	0.197(0.079)
P-value	0.072	0.006	0.019	0.026
Non-Hispanic black				
β(SE)	0.045(0.047)	-0.022(0.020)	-0.011(0.007)	0.045(0.032)
P-value	0.349	0.287	0.115	0.176
Other race				
β(SE)	0.235(0.059)	0.143(0.099)	-0.025(0.010)	0.151(0.059)
P-value	0.001	<0.001	0.020	0.022
Creatinine*				
β(SE)	0.942(0.034)	-0.142(0.147)	-0.044(0.005)	0.414(0.022)
P-value	<0.001	0.348	<0.001	<0.001
Creatinine-squared*				
β(SE)	--	0.158(0.039)	--	--
P-value		0.001		
Model R-squared	**0.4890**	**0.2012**	**0.1633**	**0.3311**

* log(10) transformed values

APPENDIX L (continued)

B. LOG(10) ADJUSTED DIETARY INORGANIC ARSENIC MODELS

	Urinary Total As*	Urinary Sum of Species As*	Urinary %MMA	Urinary DMA:MMA*
Dietary inorganic As*				
β(SE)	0.267(0.057)	0.189(0.029)	-0.015(0.008)	0.149(0.045)
P-value	<0.001	<0.001	0.083	0.004
Sex (female)				
β(SE)	0.051(0.039)	0.032(0.023)	-0.010(0.006)	0.061(0.027)
P-value	0.206	0.176	0.094	0.040
Age				
β(SE)	0.003(0.001)	0.001(0.000)	-0.000(0.000)	0.002(0.000)
P-value	0.001	0.021	0.012	0.001
BMI				
β(SE)	-0.006(0.001)	-0.004(0.001)	-0.000(0.000)	-0.001(0.002)
P-value	0.001	<0.001	0.425	0.654
Current smoking				
β(SE)	-0.088(0.036)	-0.036(0.018)	0.015(0.004)	-0.078(0.021)
P-value	0.028	0.063	0.003	0.002

APPENDIX L (continued)

B. (continued)

	Urinary Total As*	Urinary Sum of Species As*	Urinary %MMA	Urinary DMA:MMA*
Non-Hispanic white	Ref	Ref	Ref	Ref
Mexican-American				
β(SE)	0.083(0.053)	0.094(0.031)	-0.006(0.005)	0.061(0.020)
P-value	0.138	0.008	0.219	0.008
Other Hispanic				
β(SE)	0.262(0.071)	0.190(0.045)	-0.035(0.010)	0.228(0.068)
P-value	0.002	0.001	0.003	0.004
Non-Hispanic black				
β(SE)	0.099(0.058)	0.001(0.024)	-0.014(0.007)	0.069(0.038)
P-value	0.109	0.967	0.069	0.091
Other race				
β(SE)	0.366(0.081)	0.174(0.032)	-0.033(0.010)	0.196(0.058)
P-value	<0.001	<0.001	0.004	0.004
Creatinine*				
β(SE)	0.929(0.051)	-0.207(0.167)	-0.043(0.006)	0.408(0.029)
P-value	<0.001	0.236	<0.001	<0.001
Creatinine-squared*				
β(SE)	--	0.174(0.046)	--	--
P-value		0.002		
Model R-squared	**0.3731**	**0.4433**	**0.0727**	**0.2686**

* log(10) transformed values

APPENDIX M

COMPARISON OF DIETARY ARSENIC EXPOSURE AND URINARY TOTAL ARSENIC IN FOUR STUDY POPULATIONS

	Schoof Dietary Total As µg/day	Schoof Dietary Inorganic As µg/day	Urinary Total As µg/L
NHEXAS-AZ			
geo mean	54.30	7.16	9.05
median	54.46	6.77	10
min-max	14.3-225	2.2-46.0	2.35-430
ABS			
geo mean	85.47	11.21	6.50
median	76.77	9.61	2.35
min-max	15.2-1632	3.0-65.2	2.35-341
BAsES			
geo mean	32.78	5.93	25.74
median	27.2	5.23	22.63
min-max	4.82-495	0.83-62.8	1.55-1121
NHANES 2003-2004			
geo mean	41.08	6.20	8.29
median	33.99	5.59	8.2
min-max	3.70-1580	0.78-93.0	0.4-1221

APPENDIX N

COMPARISON OF UNADJUSTED URINARY SPECIATED ARSENIC MEASURES IN BASES AND NHANES

	BAsES	2003-2004 NHANES
Sum of species As		
geo mean	10.86	5.97
median	10.05	6.1
min-max	0.87-1042	3.3-89
As^{+3}		
geo mean	0.55	§
median	0.58	0.8
min-max	0.06-68.96	0.8-23
As^{+5}		
geo mean	0.48	§
median	0.48	0.7
min-max	0.10-157	0.7-8.8
MMA^{+5}		
geo mean	1.24	§
median	1.17	0.6
min-max	0.13-209	0.6-16
DMA^{+5}		
geo mean	8.05	3.71
median	7.35	4.0
min-max	0.58-672	1.2-71

Appendix N (continued)

	BAsES	2003-2004 NHANES
% MMA^{+5}		
geo mean	11.40	14.06
median	12.02	13.64
min-max	3.72-40.71	0.95-46.0
DMA:MMA		
geo mean	6.50	5.88
median	6.43	4.61
min-max	1.28-23.70	0.52-101.5

§ >60% of values below the limit of detection: geometric means not calculated.

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