ESTIMATING THE GLOBAL BURDEN OF DISEASE CAUSED BY ARSENIC IN FOOD

by

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Estimating The Global Burden of Disease caused by Arsenic in Food

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ABSTRACT

Background Arsenic is a ubiquitous, naturally occurring metalloid that poses a significant risk for human cancer and non-cancer diseases. While water consumption provides the majority of human exposure to arsenic, millions of individuals worldwide are significantly exposed through naturally occurring levels of arsenic in grains, vegetables, meats and fish, as well as through food processed with water containing arsenic.

Objectives This research estimates the global burdens of disease for bladder, lung and skin cancers as well as coronary heart disease attributable to inorganic arsenic in food.

Methods In order to determine foodborne inorganic arsenic exposures worldwide, this research uses the World Health Organization's estimates of food consumption in 13 country clusters, in conjunction with the reported measurements of total and inorganic arsenic in different foods. This research estimates slope factors for arsenic related bladder and lung cancers, and adopts the US Environmental Protection Agency skin cancer slope factor to calculate the annual risk of cancer incidence in males and females within each country cluster. Benchmark dose and reference dose for arsenic induced coronary heart disease are derived using US Environmental Protection Agency Benchmark dose modeling software.

Results The research findings show that each year across the world 9,129 to 119,176 additional cases of bladder cancer; 11,844 to 121,442 of lung cancer; and 10,729 to 110,015 of skin cancer are attributable to inorganic arsenic in food. For coronary heart disease, foodborne inorganic arsenic can cause up to 329,750 additional cases annually in USA and even higher rates in GEMS clusters with higher foodborne arsenic exposures. However, in contrast to cancer burden, there is a threshold effect resulting in no increased risk of heart disease at the expected lower bound of arsenic consumption in food.

Conclusions These estimates indicate that foodborne arsenic exposure causes a significant global burden of human disease.

Public Health Impact Estimating the global burden of disease caused by arsenic exposure in food will support policies that reduce exposure to disease promoting environmental hazards.

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1.0 INTRODUCTION

1.1 ARSENIC OVERVIEW

Arsenic is a naturally occurring metalloid that is found in both organic and inorganic forms. It is the twentieth most abundant element in the earth's crust and also gets introduced into the environment by anthropogenic activity. In the natural environment arsenic frequently exists in its inorganic form as a component of ores and is concentrated on the continental crust of earth at approximately 1.5 to 2 parts per million (Arsenic, 1977). Inorganic arsenic can exist in four valence states: -3, 0, +3 and +5, depending upon the environmental conditions. The two most common oxidation states are arsenate (e.g., As (V)) and arsenite (e.g., As (III)) that exist in oxidizing and mildly reducing conditions respectively (Duker, 2005). Valence states may interchange in accordance with the pH and the presence of other substrates (Freeman, 1993).

Inorganic arsenic often complexes with copper, lead, iron, nickel, cobalt, silver, thallium and other metals yielding more than 245 mineral forms of arsenic (Arsenic, 1977). Arsanilic acid, methylarsonic acid and dimethylarsinic acid are the most common organic arsenic compounds whereas the most common inorganic compounds exist as arsenic trioxide, sodium arsenite, and arsenates (e.g., lead arsenate) (WHO, 2000).

In sedimentary rocks and soils, arsenic is found in combination with sulfides, hydroxides, manganese and iron (Rehman et al., 2012). Generally the soil concentrations of arsenic are 5 to 6 ppm approximately, but they can vary between 0.2 to 40 ppm across various geographic regions (Jones, 2007). In the vicinity of copper smelters, the levels of arsenic in soil have been found ranging from 100 to 2500 mg/kg (Diaz-Barriga, 1993).

Volcanic activity is the primary source of natural airborne arsenic whereas the smelting of metals, fuel combustion and pesticide use form its major anthropogenic sources. In the United States, the average level of arsenic in ambient air ranges from <1 to 3 ng/m^3 in remote areas and 20 to 30 ng/m^3 in urban areas (ATSDR, 2007). Climate and geology influence the level of arsenic in the ground water (Bhattacharya, 2007). Arsenic exists at high levels in sediments of rivers and lakes. Various pockets of the US including New England, Maine and areas in the West including Arizona, New Mexico, Nevada and Utah have high levels of arsenic (Brown, 2002).

At the global scale countries like Bangladesh, India, Taiwan, Mexico, China, Argentina and Chile have the highest arsenic concentration in the groundwater (ranging to low parts per million) in many areas (WHO, 2000). High levels of arsenic and other metals in natural groundwater sources pose a major threat to public health worldwide. About 45 million people in Bangladesh are exposed to disease-inducing high levels of arsenic in drinking water (more than the WHO guideline of 10 μg/L) (Flanagan et al., 2012). Factors like pH, reducing and oxidizing conditions composition of the solution and temperature can determine the amount of arsenic that gets solubilized into the ground water from the bedrock (Focazio et al., 1999; Nordstrom, 2002). Additionally, high levels of groundwater arsenic are directly influenced by specific environmental conditions. Closed basins in arid to semi-arid climates and strongly reducing

aquifers composed of low sulfate alluvial sediments are typically inducing of higher levels of arsenic in ground water. Dissimilatory arsenate-reducing prokaryotes can reduce and/or oxidize arsenic containing soils and water - thus enhancing the levels of arsenic in the ground water (Focazio et al., 1999).

1.2 ARSENIC EXPOSURE THROUGH FOOD

While isolated industrial sources provide significant exposures, the vast majority of individuals are chronically exposed to arsenic by ingestion of contaminated food or drinking water. According to a recent WHO background document on arsenic in drinking water (WHO, 2011), water consumption provides the majority of arsenic exposures and exposure may be more harmful due to all of the arsenic in the water being in a toxic form.

Groundwater is a major source of drinking water in many parts of the world. Concentrations of arsenic in groundwater are usually less than 10 μg/L, but they can reach much higher levels in some areas (Smedley et al., 2002). Arsenic concentrations are lower in surface waters than groundwater. All the arsenic in drinking water is in its inorganic form. Arsenic is present mainly as arsenate in oxygenated conditions, such as found in most surface waters. However arsenite can be the dominant species in some groundwaters under certain reducing environmental conditions. For instance in Bangladesh, the arsenic in ground water is approximately 97% arsenite (Postma et al., 2007).

Naturally occurring levels of arsenic in vegetables, grains, meats and fish, as well as in food processed with water containing arsenic (e.g., cooking rice), present significant exposures

affecting many millions of individuals worldwide (Yost et al., 1998). It is unlikely that there are individuals who are not exposed to some level of arsenic in food. However, the extent of human exposure to toxic forms of arsenicals in foods is difficult to estimate and highly variable due to the natural distribution of arsenic in soils and water (WHO, 2011). In addition, inert, non-toxic organic arsenicals found in seafood and food products made from seaweed can obscure the estimates of daily intake of toxic arsenicals.

1.2.1 Arsenic species in food

Arsenic levels in food are usually described as total arsenic content, which is the sum of all arsenic species. Data on arsenic species is extremely important as different types of foods contain different arsenic species with varying toxicities. However, inorganic arsenic is the species of greatest health relevance (JECFA, 2011).

1.2.1.1 Inorganic arsenic species

Inorganic arsenic species in the environment predominantly include the +3 or +5 oxidation state, present as thio- complexes or, primarily as the oxoanions arsenite and arsenate. In food samples, inorganic arsenic is often reported as arsenite and arsenate as these are the analytes that are actually measured. However the arsenic species are likely non-covalently bound to thiol groups in peptides or proteins in the food itself. Since the total arsenic content of food products of terrestrial origin is generally low, the overall inorganic arsenic content is low as well with the exception of rice. Rice contains significant amounts of inorganic arsenic with concentrations

often between 0.1 to 0.4 mg arsenic/kg dry mass or higher (Sun et al., 2008; Meharg et al., 2009).

Total arsenic content in fish and other seafood is typically high, i.e., 2 to 60 mg arsenic/kg dry mass (SCOOP, 2004; Julshamn et al., 2004) and the levels of inorganic arsenic are <0.2 mg arsenic/kg dry mass (Edmonds et al., 2003; Sloth et al., 2005; Sirot et al., 2009). However, edible marine alga hijiki (*Hizikia fusiforme*, also called hiziki) has exceptionally high inorganic arsenic concentrations (as arsenate) of >60 mg/kg (FSA, 2004) and blue mussel (*Mytilus edulis*) can have inorganic arsenic concentrations up to 30 mg/kg dry mass (Sloth et al., 2008).

1.2.1.1 Organic arsenic species

The major organoarsenic compounds found in foods are arsenobetaine, arsenosugars and arsenolipids. More than 50 organoarsenic compounds have been reported in marine organisms that are used as food items. Most of these usually occur only at trace levels. Arsenosugars and arsenolipids are mainly metabolized in humans to dimethylarsinate, but no specific information is available regarding their toxicity. Risk to human health due to arsenosugars, arsenolipids, methylarsonate and dimethylarsinate has not been characterized.

Arsenobetaine is the major form of organic arsenic found in fish and most seafood and is widely assumed to be of no toxicological concern (EFSA, 2009). Arsenobetaine occurs in minor levels in some mushroom species (Francesconi et al., 2002) and in marine algae (Nischwitz et al., 2005). Arsenosugars are the dominant arsenic species in algae. Arsenobetaine is also found in freshwater organisms at much lower levels than those found in marine samples (<0.1 mg arsenic/kg dry mass) (Slejkovec et al., 2004; Schaeffer et al., 2006). Arsenobetaine can be

present at higher concentrations in farmed freshwater fish (aquaculture products) because they are provided with feed containing marine ingredients (Soeroes et al., 2005).

Major arsenical constituents of marine algae are arsenosugars (2 to 50 mg arsenic/kg dry mass). Arsenosugars are also found at significant concentrations in animals feeding on algae (e.g., mussels and oysters; 0.5 to 5 mg/kg dry mass) (Francesconi et al., 2002; EFSA 2009) and at lower concentrations in many other marine organisms.

Arsenolipids are lipids that contain arsenic. The structures of some of these arsenolipids have only recently been elucidated although arsenolipids in fish were first reported in the late 1960s. Six arsenic-containing fatty acids are found in cod-liver oil (EFSA, 2009; Rumpler et al., 2008). Arsenolipid content has been found to vary between about 4 to 12 mg arsenic/kg of oil (Schmeisser et al., 2005; Taleshi et al., 2008) in the fish oils examined so far.

Among other organoarsenic species, methylarsonate, dimethylarsinate, trimethylarsine oxide, and tetramethylarsonium ions are often found in organisms generally at low concentrations (<0.5 mg arsenic/kg dry mass) (Francesconi et al., 2002). Arsenocholine also occurs commonly, but generally at modest levels, in marine organisms (typically <0.2 mg arsenic/kg dry mass) (EFSA, 2009).

1.3 ARSENIC TOXICOKINETICS

Inorganic arsenic (As (III) or As (V)) has a complex metabolism and is readily absorbed through the gastrointestinal tract (NRC, 2013; WHO, 2000). Human tissues, blood, and urine contain a

mixture of arsenic metabolites that vary in acute and chronic toxicity (NRC, 2013). Rate and extent of methylation varies between different species as well as among humans (Hughes et al., 2011). In mammals arsenic is transported to the liver after being absorbed through the gastrointestinal tract. Arsenic gets transported and distributed throughout the body through blood and is rapidly excreted through urine (Thomas et al., 2001). Human autopsy data indicate that the highest absolute amounts of arsenic are found in lungs, kidneys, and skeletal muscle (WHO, 2000); however, high levels of arsenic accumulate in skin, nails and hair.

Following low to moderate exposures to arsenic, the absorbed portion of pentavalent arsenic gets reduced to its trivalent form (Vahter, 2002). Metabolism of inorganic arsenic (presented in detail below) produces various arsenic species that differ in toxicity, rates of elimination and tissue distribution (NRC, 2014). The mode of action of inorganic arsenic might be influenced by the type of tissue, and exposure factors. The rate of uptake, intracellular distribution and the expulsion rate of different arsenic metabolites varies with the tissue type. This in turn leads to variation in toxicity of the different metabolites (Dopp et al., 2010). Arsenic methylation efficiency varies extensively among tissues with liver having the highest levels of the arsenic (3) methyl-transferase enzyme (AS3MT; Kobayashi et al., 2007). Limited human data exists to enable physiology-based pharmacokinetic modeling for predicting the concentrations of inorganic arsenic in specific tissues (El-Masri et al., 2008). Some evidence exists for the formation of arsenical thiols (Fricke et al., 2005). However, as thiols tend to be oxidized upon elimination, there is little evidence of its detection in human urine (Raml et al., 2007; Currier et al., 2013).

Metabolism of arsenic involves sequential steps of reductive and oxidative methylation. The key components of arsenic metabolism in this pathway are S-adenosyl methionine (SAM) and AS3MT that respectively serve as the methyl donor and the principal enzyme for methylation of inorganic arsenic (Hughes et al., 2011; Gamble et al., 2012). Hepatocytes, with the highest AS3MT content, take up trivalent arsenic and metabolize it through oxidative methylation to methylarsonic acid (MMA(V)) using SAM as a co-substrate. The methylation of MMA(III) by AS3MT yields dimethylarsinic acid (DMA(V)), which is considerably less toxic than inorganic As (III), As (V), or MMA(III). The ratio of MMA(III) to DMA(V) is most critical in arsenic metabolism, because MMA(III) is the most biologically active species. Sulfhydryl groups (-SH) on AS3MT may act as the reductant in the absence of GSH (Naranmandura et al., 2006). Most species methylate inorganic As species to varying degrees in a process commonly thought to involve alternate reduction and oxidative methylation reactions (Hall et al., 2012). Challenger proposed the widely accepted classical pathway (Figure 1) for methylation of inorganic arsenic (Challenger, 1947 and 1951; Hughes et al., 2011).

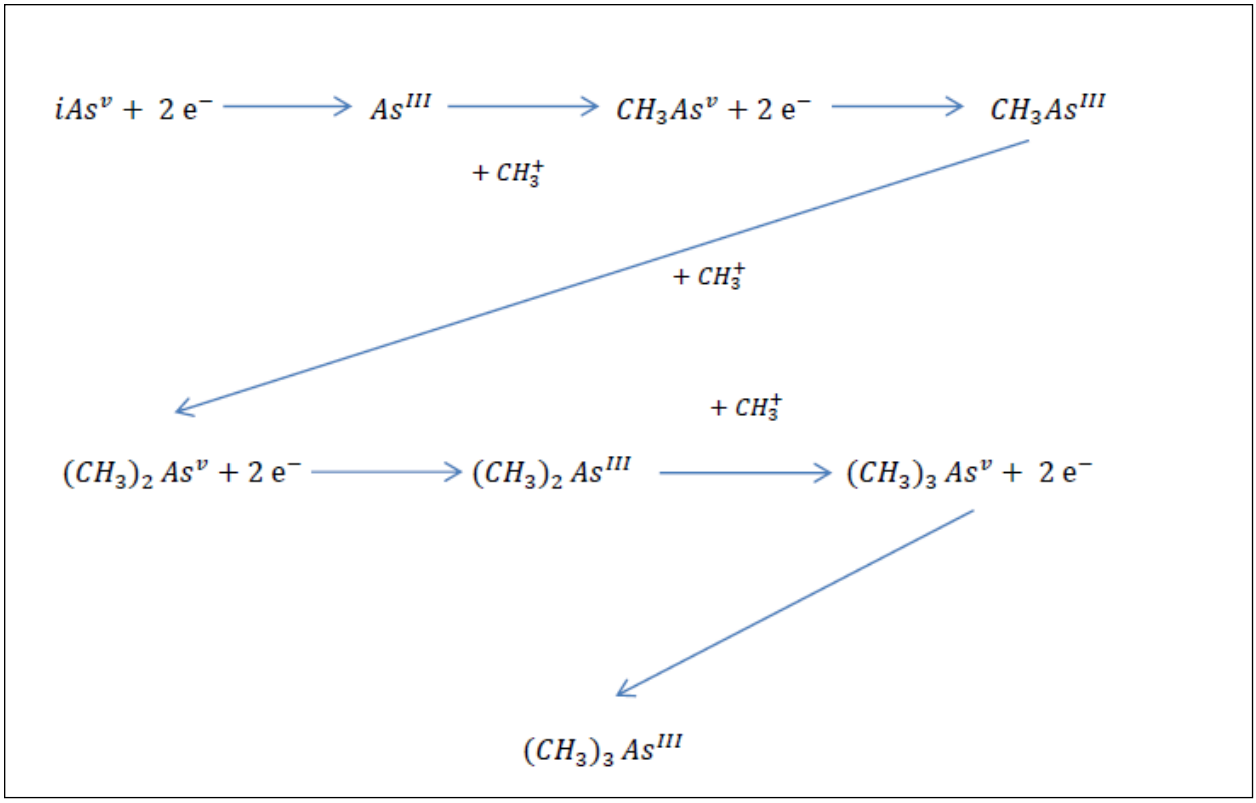


Figure 1 - Oxidative methylation pathway for inorganic arsenic in mammals

(Adapted from Hughes et al, 2011 based on Challenger, 1951)

It has recently been reported that pentavalent arsenic metabolites are hardly metabolized in mammals (Rehman et al., 2012). DMA (V) was found to be readily excreted in the urine in an unchanged form after oral and parenteral administration in mice and rats. Similarly, in goats, sheep, mice and humans, the orally administered MMA(V) was excreted in urine in its unmodified form. These findings suggested that the classical pathway should be reconsidered.

Hayakawa proposed an alternative mechanism where a thiol such as glutathione (GSH) or other endogenous reductants (like thioredoxin) can catalyze the reduction of arsenic (Hayakawa et al., 2005). As per this scheme, the oxidation of trivalent methylated metabolites (MMA(III) and DMA(III)) produces pentavalent metabolites (MMA(V) and DMA(V)).

1.4 HEALTH EFFECTS OF ARSENIC

Inorganic arsenic exposure causes multiple adverse health effects – both cancerous and non-cancerous - in humans. The International Agency for Research on Cancer (IARC) has concluded that arsenic in drinking water causes skin, bladder and lung cancer; and that there is limited evidence of it causing kidney, liver and prostate cancer (Straif et al., 2009). IARC has classified arsenic as a Group 1 carcinogen (IARC, 2012). Heart disease (myocardial infarction), respiratory disease, hyperkeratosis, peripheral vascular disease, and possibly hypertension are the most common non-cancer causes of mortality and morbidity. Figure 2 depicts the influence diagram by which arsenic accumulates in foods and then contributes to adverse human health effects.

Inorganic arsenic and some organic arsenicals consumed have wide ranging pathogenic effects that contribute to dose-dependent increased risk for disease. The exact mode of action (MOA) for toxicity from chronic exposures through food or drinking water for the disease endpoints shown in Figure 2 remains unresolved. Hughes et al. (2011) discuss how the most appropriate dose response risk assessment model (e.g., linear or non-linear) is still subject to debate and that the different disease endpoints, even cancers, may have thresholds for arsenic risk. Figure 2

shows that several modes of action may be responsible for adverse arsenic effects; however, the modes of action might not be independent and more than one might contribute to different diseases. In addition, the pathogenic actions and MOA are dictated by the form of arsenic (e.g., valence state, inorganic versus organic, and type of organic arsenical).

General nutritional status of the exposed individual can also influence the toxic risk, since folate-dependent one carbon metabolism of inorganic arsenic can both create more toxic methylated species and enhance the elimination of ingested arsenic to reduce toxicity (Hall et al., 2012). Folate, selenium and vitamin status greatly influence the epigenomic impact of arsenic that is increasingly being realized as a major mechanism for disease promotion in adults and imprinting for disease risk in-utero. Nevertheless, foodborne exposure to inorganic arsenic in non-occupationally exposed adults is undeniably important. The exposure through rice and other food crops that bio-accumulate arsenic, especially in areas with low arsenic levels in water, pose a critical risk (Sayarath, 2009).

1.4 GLOBAL BURDEN OF DISEASE (GBD) DUE TO ARSENIC

The incidence and/or prevalence of morbidity, disability and mortality associated with acute and chronic manifestations of disease can be defined as burden of disease (WHO, 2006). GBD is a widely accepted parameter that provides a frame of reference for comprehensive analysis of health gaps. It relies on the use of all available mortality and health data by appropriate methods to confirm the comparability and consistency of estimates of demographic and epidemiological importance worldwide. The World Health Organization has acknowledged the need for an

accurate estimation of the extent of foodborne illness to help ensure public health security and to enable socioeconomic development worldwide (Kuchenmuller et al., 2009). Scientific evidence allows policy makers to prioritize the allocation of limited resources to improve public health in the most efficient and effective manner; additionally, it allows informed decisions to be made to evaluate the current food safety measures and develop new food safety standards. Knowledge of the burden of disease attributable to food toxins helps to assess the cost-effectiveness of interventions and to quantify the disease burden in monetary costs.

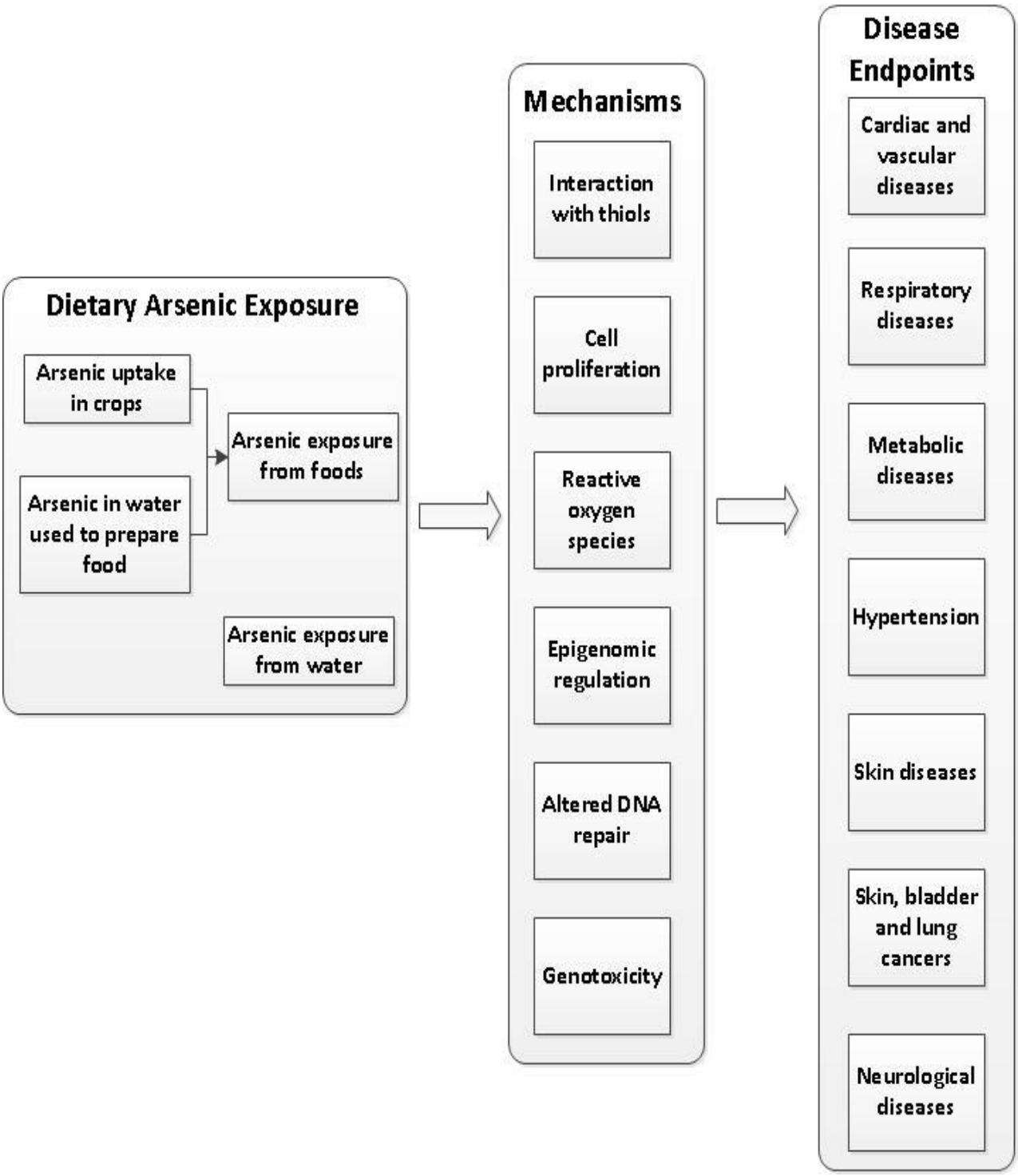


Figure 2 **- Foodborne arsenic and disease pathways in humans**

WHO and other agencies have extensively used the GBD to describe the global, regional and national burden from diseases (Murray et al., 1996; Kuchenmuller et al., 2009). Other composite measures of population health status such as the disability-adjusted life years (DALY) help to define the overall burden of disease. DALY is a time-based measure that "combines years of life lost due to premature mortality and years of life lost due to time lived in disability or states of less than full health" (Murray et al., 1996).

This research was conducted as part of the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) efforts to estimate the GBD from foodborne chemical exposures, including dietary inorganic arsenic exposure, in response to the increasing global interest in health information and addressing a bigger need to fill the current data gap. A partial risk assessment was made previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) who reviewed the provisional tolerable weekly intake (PTWI) of inorganic arsenic with an emphasis on the speciation and occurrence of inorganic arsenic in food (JECFA, 2011). JECFA (2011) indicated that organic forms of arsenic present in seafood needed different consideration from inorganic arsenic and that there have been no reports of ill-effects among populations consuming large quantities of fish resulting in relatively high organoarsenic intakes.

In addition, the human health risks in European countries from foodborne arsenic were assessed by the EFSA Panel on Contaminants in the Food Chain (EFSA, 2009). However, the global burden of cancers caused by foodborne arsenic exposure has not been investigated, nor the extent of inorganic arsenic content in different diets worldwide.

This research focuses on estimating human burden of disease caused by foodborne arsenic exposures. The emphasis is on adverse effects associated with *inorganic* arsenic exposure, because it is not clear that foodborne organic arsenic exposure causes human health risk.

2.0 METHODS

Risk assessment can be defined as the process of estimating the magnitude and the probability of a harmful effect to individuals or populations from certain agents or activities (Omenn, 2000). It involves systematic scientific evaluation of potential adverse health effects resulting from human exposures to hazardous agents or situations (NRC, 1983; 1994). Estimation of risk mainly involves four steps: hazard identification, dose-response analysis, exposure assessment, and risk characterization.

2.1 HAZARD IDENTIFICATION

Hazard identification is the process of determining whether exposure to an agent can increase the incidence of a particular health condition (Liu et al., 2010). Arsenic exposure is associated with an increase in the incidence of bladder, lung and non-melanoma skin cancers in humans. IARC has classified arsenic as a Group 1 carcinogen (IARC, 2012). Cardiovascular disease is arguably the most important non-cancer disease risk from environmental arsenic exposures, since cardiovascular disease is the most common cause of death worldwide and even a modest increased relative risk from arsenic exposure translates to very large numbers of excess cases. Arsenic exposure at levels present in food is associated with coronary artery disease (CAD), myocardial infarctions (MI), and peripheral vascular disease (PVD, a form of ischemic disease).

The mortality risk from cardiovascular disease was indeed found to be increased by 22% to 35% for arsenic exposure levels of 12 ppb to 148 ppb in a recent prospective study in approximately 12,000 subjects in Bangladesh (Chen et al., 2011).

2.2 QUANTITATIVE CANCER RISK ASSESSMENT

The two key parts of quantitative risk assessment are *dose-response* (or *health effects*) *analysis* and *exposure assessment*. Dose-response analysis involves characterizing the relationship between the dose of an agent and incidence of the disease associated with an exposure to this agent (Liu et al., 2010). For a toxic agent, exposure assessment includes an estimation of the dose i.e. the intensity, frequency, and duration of human exposures. Specifically, this step seeks to determine the effects of exposure to toxic agent on the exposed individuals' health (Liu et al., 2010).

In this research, the dietary arsenic exposure was multiplied by the cancer potency factor (slope factor) for a given cancer endpoint in order to assess the quantitative cancer risk for a given population. The global estimate for burden of a particular arsenic-induced cancer was then obtained by summing across different populations.

2.2.1 Dose-response assessment

Dose response assessment involves quantitative evaluation of the toxicity information in order to characterize the relationship between the dose of the contaminant (administered or received) and the incidence of adverse health effects in the exposed population. This process is used to derive

quantitative toxicity values (i.e., cancer slope factors or reference doses) for comparison to environmental exposure levels (US EPA, 2012; Wignall et al., 2014) and can be used to estimate the incidence or potential for adverse effects as a function of human exposure to the agent.

2.2.1.1 Cancer slope factor approach

Risk of cancer associated with exposure to a carcinogenic or potentially carcinogenic substance is estimated by the use of cancer slope factors. A slope factor is an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent by ingestion (USEPA IRIS, 1998). It provides an estimate of the probability of a response per unit intake of a chemical over a lifetime and is usually expressed in units of proportion of a population affected per mg of substance/kg body weight-day.

This approach is generally reserved for use in the low-dose region of the dose-response relationship, i.e., for exposures corresponding to risks less than 1 in 100. The Incremental Lifetime Cancer Risk can be estimated by multiplying the cancer slope factor by the chronic daily intake. The chronic daily intake represents the dose over a lifetime and is expressed in mg/kg-day.

For the risk assessment considered here, cancer slope factors for bladder cancer and lung cancer were derived using data adapted from Morales et al. (2000) where the authors provided a risk assessment based on re-analysis of data originally reported in early studies from arsenic-endemic region of southwestern Taiwan (Chen et al., 1988, 1992; Wu et al., 1989). The paper by Morales et al. (2000) examined model sensitivity and calculated the risk of cancer mortality using 10 models. Table 8 of this paper (Appendix B) provides the concentration of arsenic in drinking

water (μg/L) estimated to cause bladder or lung cancer in 1% of males and females in a cohort in southwestern Taiwan.

The derived cancer potency factor was transformed to be relevant to human doses by assuming a daily consumption of 2 liters of water per adult in the southwestern Taiwanese population. These transformed cancer slope factors were multiplied by arsenic exposures in food for different populations worldwide. For skin cancer caused by inorganic arsenic, the slope factor was adapted from the United States EPA IRIS database (US EPA IRIS, 1998). The dietary arsenic exposures were collected for all countries of the world using the GEMS cluster diets database (WHO GEMS, 2006) to estimate the burden of cancer caused by foodborne arsenic in each nation as described in Section 2.2.2.

2.2.1.2 Reference dose approach for non-cancer disease endpoints

Reference dose (RfD) is an estimate of a daily exposure to an agent that is assumed to be without adverse health impact in humans (Cassarett and Doull's, 2010). It is the estimated value of the daily oral exposure of a population to a toxin that is likely to be without an appreciable risk of deleterious effects during a lifetime. Uncertainty associated with RfD can span several orders of magnitude. It can be derived from a no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), or benchmark dose (BMD), with uncertainty factors generally applied to reflect limitations of the data used and to provide a margin of safety that accounts for variability in susceptibility. The RfD is generally used in EPA's non-cancer health assessments.

For the estimation of a RfD value for coronary heart disease caused by foodborne arsenic, this research relied on the benchmark dose modeling approach. A mathematical model to estimate the

lower confidence bounds on a predetermined level of risk (the "benchmark dose" (BMD)) was proposed by Crump (1984). BMD is the central estimate of the dose or concentration that produces a predetermined change in the response rate of an adverse effect (USEPA: Risk Assessment). The predetermined change in response is called the benchmark response (BMR).

The BMD approach was proposed as a means of avoiding many of the disadvantages of the traditional no observed adverse effect level (NOAEL) approach to determine the point of departure (POD) from animal toxicology data for use in human health risk assessments. However, NOAEL strictly depends on the selected dose, as well as dose spacing and the sample size from the study from which the critical effect has been identified. Additionally, the NOAEL approach does not consider the shape of the dose-response (Davis et al., 2011). Shallow dose–response, small sample sizes, wide spacing of experimental dosage levels, or more than the typical number of dose levels are the major factors that highlight the discrepancies between the benchmark dose and the NOAEL. These features tend to make determination of the NOAEL more problematic (usually higher) and the confidence limits around the maximum likelihood estimate broader (resulting in lower BMDs).

BMD modeling defines a point of departure (POD) that is largely independent of study design. BMD is estimated by fitting of various mathematical models to the observed data. Benchmark dose methods involve analyzing each endpoint's dose response separately. In accordance with the EPA's draft Benchmark Dose Technical Guidance (US EPA, 2012), dose-response modeling was conducted using the US EPA's benchmark dose (BMD) software (BMDS, version 2.4) to identify the potential points of departure (PODs) for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$). Selecting the

BMR(s) involves making judgments about the statistical and biological characteristics of the dataset and about the applications for which the resulting BMDs/BMDLs will be used. Typically, BMR near the low end of the observable range is selected as the basis for obtaining BMDs and BMDLs. These BMD and BMDL values serve as potential PODs to derive quantitative estimates below the range of observation and to use for comparisons of effective doses corresponding to a common response level across chemicals, studies, or endpoints.

2.2.1.3 BMD modeling methodology

BMD analysis can be based on any dose-response study that (i) shows a graded monotonic response with dose, and (ii) has the minimum data set for calculating a BMD showing a significant dose-related trend in the selected endpoint(s) (USEPA BMDS, 2012). For a better estimate of the BMD and a shorter confidence interval, studies with one or more doses near the level of the BMR are preferred.

Studies that do not have a NOAEL (i.e., in which all the dose levels show changes compared with control values) are useful for BMD analyses, as long as the lowest response level is not much higher than the BMR.

In any toxicological experiment, various end points or observations can be monitored (USEPA BMD, 2012). For the purpose of BMD modeling, endpoint data can be classified into one of three categories viz. dichotomous (quantal), continuous and categorical. The clinical signs such as mortality due to coronary heart disease can be expressed as a ‘yes’ or ‘no’ response. These kinds of data are called dichotomous data. Dichotomous data are quantal data where an effect for an individual may be classified by one of two possibilities.

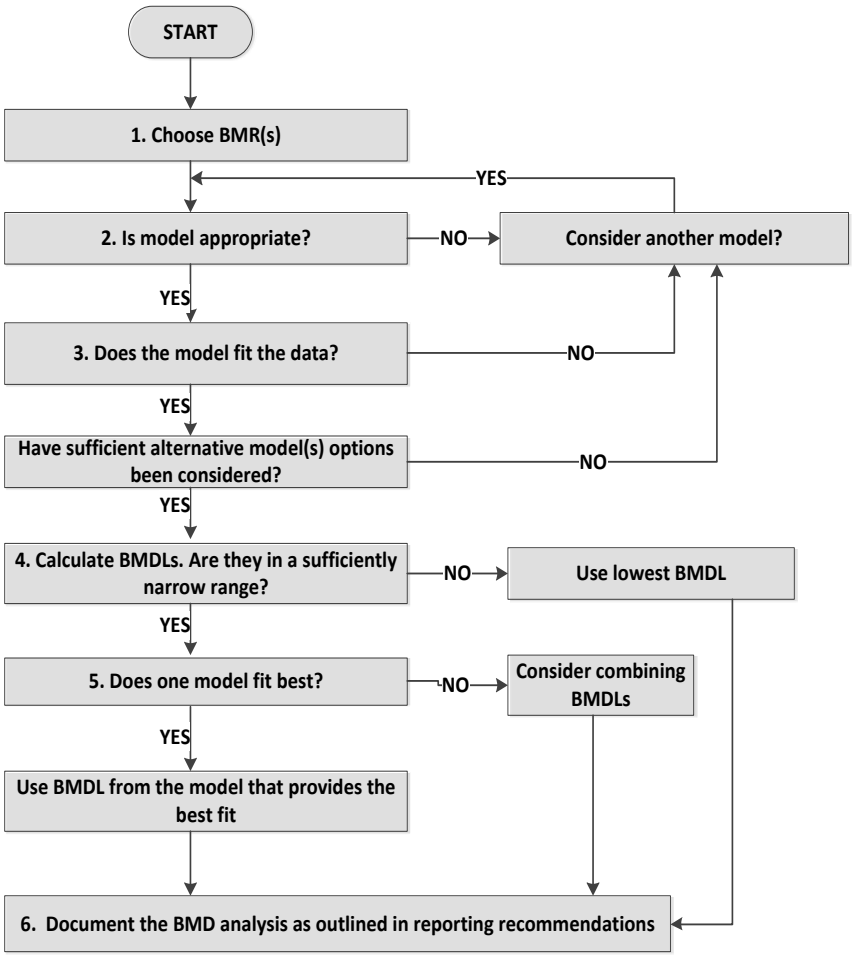


Figure 3 - Benchmark Dose Decision tree*

*(Adapted from USEPA BMD Technical Guidance document, 2012)

In contrast, the percentage increase in surface area of atherosclerotic lesions is expressed as a continuum. Therefore, each animal will have an exact measurement of a value and the group data

are usually expressed as mean and standard deviation. These data are called continuous data (USEPA BMD, 2012). For each treatment group, the data are expressed as the sample size, mean response and standard deviation or standard error.

For categorical data, the responses in the treatment groups are often characterized in terms of the severity of effect (e.g., mild, moderate, or severe histological change).

Data from two distinct studies (one prospective cohort epidemiological study and another animal study) were used to arrive at BMDL values.

Studies selected for BMD calculation

Rationale for study selection and endpoint selection

The most representative epidemiological study for arsenic related coronary heart disease (CHD) incidence (Moon et al., 2013) was selected based on the literature search presented in Chapter 3.

BMD approach begins by identifying a criterion for adverse effect. In most analyses, the criterion for adverse effect has been the manifestation of disease in a non-exposed population (Jacobson et al., 2002). Epidemiological evidence for an association between arsenic exposure and CHD is stronger than any other non-cancer disease (NRC, 2013). Hence CHD incidence was considered the best disease endpoint for the purpose of deriving a benchmark dose for arsenic.

Primary data from a mouse study was also analyzed using the BMD software to compare the output to that from the epidemiological data. Atherosclerosis is the primary mechanism in the etiology of arsenic-related ischemic heart disease. Arsenic-induced atherogenesis has been demonstrated in a genetic mouse model of atherosclerosis after low to moderate exposure to

arsenic (NRC, 2013; Lemaire et al., 2011). Earlier animal studies have reported that water arsenic exposures accelerate and exacerbate atherosclerosis in the apolipoprotein E-knockout ($ApoE^{-/-}$) mouse model for atherogenesis (Srivastava et al., 2007, 2009). In these studies, atherogenesis was induced by high level arsenic exposure (50 ppm) alone, without the high fat diet normally used to induce atherosclerosis in this model (States et al., 2009). These responses were clearly linked to the disease predisposition of the mice that appears to be aggravated by the arsenic exposure.

Primary data from the laboratory of our collaborator Dr. Koren Mann (McGill University, Montreal, Canada) was analyzed for use in this research for BMD modeling (Table 2). This is the first animal study to examine clinical/ pre-clinical modality directly related to coronary heart disease conducted with an exposure range that is directly relevant to human exposures.

Study Information

Epidemiological study for BMD modeling: (Moon et al., 2013)

This recent prospective cohort study derives epidemiological data from the Strong Heart Study (SHS). SHS is a study of cardiovascular disease and its risk factors among American Indian men and women in three geographic areas: an area near Phoenix, Arizona; the southwestern area of Oklahoma; and western and central North and South Dakota (SHS, 2006). Moon et al., (2013) was based on arsenic measurements from the urine samples of 3575 men and women aged 45 to 74 years at baseline between 1989 and 1991 and were actively followed through 2008. Comparing the highest and lowest quartiles of arsenic concentrations (>15.7 µg/g vs. <5.8 µg/g creatinine), the hazard ratios for cardiovascular disease, coronary heart disease, and stroke

incidence after adjustment for socio-demographic factors, smoking, body mass index, and lipid levels were 1.32 (CI, 1.09 to 1.59; P for trend=0.002), 1.30 (CI, 1.04 to 1.62; P for trend=0.006) and 1.47 (CI, 0.97 to 2.21; P for trend=0.032) respectively (Moon et al., 2013). Data on the total number of exposed individuals and total incident cases in each arsenic exposure quartile was abstracted from this paper for BMD analysis (Table 1).

Mouse Data for BMD modeling: (Primary data)

The detailed methodology for in vivo mouse exposure and characterization of atherosclerotic lesions are described in detail in Chapter 5.

Dose response data for BMD analysis

Dose response data from Moon et al. (2013) and primary mouse data are presented in the Tables 1 and 2 below.

Table 1 - Coronary heart disease incidence endpoints by urine arsenic concentrations (μg/g creatinine)

Urinary arsenic level (μg/g Creatinine) (Median)	Number of people per dose category	Effect (number of cases)
4.2	896	202
7.5	893	206
12.4	892	197
21.8	894	241

(Data for BMD analysis)

Choice of BMR

BMR level of 1% extra risk was used to define BMD for dichotomous data from the epidemiology study relating arsenic to coronary heart disease incidence. Traditionally BMR levels of 1, 5 and 10% are used for BMD analysis of dichotomous data. The 10% response level has customarily been used for comparisons because it is at or near the limit of sensitivity in most cancer bioassays and in non-cancer bioassays of comparable size (USEPA BMD, 2012). For epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels and 1% extra risk is often used as a BMR

(USEPA BMDS, 2012). BMR of 1% was selected in this analysis as it would reflect predetermined level of the slightest increase in risk of coronary heart disease.

Table 2 - Percentage of atherosclerotic lesion area relative to total aortic sinus area in mice exposed to 0-200 ppb of arsenic in water

Dose (ppb) As in water	Number of animals exposed	Mean	Standard Deviation
0	8	0.46	0.91
10	5	3.05	1.21
50	5	4.42	2.69
100	6	8.25	1.48
200	8	15.56	4.81

For continuous data, it is recommended that the BMD (and BMDL) corresponding to a change in the mean response equal to one control standard deviation (SD) from the control mean always be

presented for comparison purposes (USEPA BMD, 2012). Accordingly, BMR level of one control SD was used to derive BMD for continuous data obtained from mice study for surface area of atherosclerotic lesions. This value was selected as it serves as a standardized basis for comparison, akin to the BMD corresponding to 10% extra risk for dichotomous data.

Selecting a Family or Families of Models

Nature of the endpoint measurement (i.e., if it is dichotomous or continuous) and the experimental design used to generate the data are critical for the initial selection of a group of models to fit to the data. For dichotomous variables, probability density models whose predictions lie between 0 and 1 for any possible dose, including 0 (like Logistic, Probit, Weibull, Multistage, Log-Logistic) are used.

In the case of continuous variables linear model, polynomial model and power models are typically considered if the BMR is expressed as – (i) a change in the mean response, (possibly as a fraction of the control mean), (ii) a fraction of the range of the response (when there is a clear maximum response), or (iii) a fraction of the standard deviation of the measurement from untreated. The Hill model can also be considered, however, it is used only when the biological response being studied is known to be receptor-mediated.

Additionally, model selection is also influenced by certain constraints on the models or their parameter values. The number of parameters that affect the overall shape of the dose-response curve generally cannot exceed the number of dose groups. EPA BMDS guidance document (EPA BMDS, 2012) suggests modeling multiple endpoints simultaneously. The need to specify a

small set of models for BMD computation is prevented by the diversity of possible endpoints and shapes of their dose-responses for different agents.

Computation of BMD and BMDL

Goodness of fit test statistic

Selection of a fitted model enables the selection of the model that provides an adequate description of the data, especially in the region of the BMR (USEPA BMDS, 2012). A global goodness-of-fit measure, usually a *p*-value is available in the fitting methods. These measures quantify the degree to which the predicted means of the dose-group differ from the actual means, relative to the expected means variation. Small *p*-values reflect a poor fit of the model because they represent the unlikelihood of the goodness-of-fit statistic to achieve an extreme value if the data were actually sampled from the model. Since *p*-values are estimated under the assumption that the different models are correct, they cannot be compared from one model to another and can only identify those models that are consistent with the experimental results (USEPA BMDS, 2012).

Use of **Akaike's Information Criterion (AIC)** is recommended for comparison of models and selection of the model to use for BMD computation. Mathematically, AIC is defined as Equation (1):

$$AIC = 2p - 2L \quad \text{Equation (1)}$$

where L is the log-likelihood at the maximum likelihood estimates (MLEs) and p is the number of parameters estimated (Akaike, 1973). All else being equal, lower AIC values are preferred.

Although the comparison of models from different families (that use a similar fitting method) by AIC values is not exact, they can provide useful guidance in model selection (USEPA BMDS, 2012).

Scaled residuals of interest is an indication of agreement between the model and the observations in the region of particular interest (i.e., the area where the response of interest (the benchmark response (BMR)) is found) (USEPA BMDS, 2012). Mathematically, scaled residuals is defined as Equation (2):

$$Scaled\_residuals = \left(\frac{Observed\_value - Expected\_value}{Std\_errors} \right) \quad \text{.....Equation (2)}$$

Scaled residuals are residuals that have been standardized by dividing by their standard errors (SE), i.e., observed minus predicted response divided by SE. Chapter 5 describes the detailed steps of BMD computation.

2.2.2 Exposure assessment

Exposure to arsenic via food depends on the concentration of arsenic in individual foods and the rate of consumption of these food items. The range of inorganic arsenic content, including a range of uncertainty and variability for different food groups that represents content in crops worldwide, was adapted from values in the literature (Schoof et al., 1999; JECFA, 2011; USEPA IRIS, 1998) to derive the mean portion of inorganic arsenic relative to the total food arsenic.

Using a common range of arsenic content for food crops grown in different parts of the world has the advantages of demonstrating the effect of dietary patterns on arsenic exposure via food,

and allowing uniformity in calculations across all nations. The range of bioavailability for the inorganic arsenic content of various foods was assumed to be 50% to 100% to take into account a factor of uncertainty. Thus for each cluster of countries, a lower and an upper bound value of inorganic arsenic content at 50% and 100% bioavailability respectively, was estimated.

To estimate the total bioavailable inorganic arsenic in the diet worldwide, these exposure assessment calculations were then consolidated for each relevant population, across all of the different foods consumed in different proportions. The GEMS Food Consumption Cluster Diets database (WHO GEMS, 2006) was used to gather information on the dietary patterns (amounts of specific foods consumed) in different parts of the world, as it divides the world into 13 clusters of countries based on dietary similarities. The GEMS database uses data from FAOSTAT to divide the countries of the world into 13 clusters on the basis of similarities in dietary pattern. In the final step, the populations of individual nations across each of the GEMS cluster were summed to estimate the global population.

2.2.3 Assumptions

The dose response assessment was based on the following major assumptions:

(i) The southwestern Taiwanese population that provides the dose-response data (Morales et al., 2000) used for estimation of the cancer potency factors are reasonably representative of global populations in terms of adverse effects of arsenic. This allowed the same cancer potency factor to be applied in other parts of the world;

(ii) The dose-response curves for arsenic-induced cancers can be linearized and driven through (0, 0);

(iii) The average human consumption of water per day is 2 liters (Morales et al., 2000);

(iv) The toxicity of inorganic arsenic exposure in drinking water is equal to that of inorganic arsenic exposure in food;

(v) The slope factors for arsenic-related bladder cancer and lung cancer, would not change appreciably as a result of infections or co-exposures in the Taiwanese population from which Morales et al. (2000) derived the data.

The primary assumption in the exposure assessment is that the values reported in literature for total foodborne exposure to arsenic and the proportion of inorganic arsenic in different foodstuffs (Schoof et al., 1999; JECFA, 2011; USEPA IRIS, 1998) are reasonably accurate. In addition, it is assumed that the rough upper and lower bounds for bioavailability of inorganic arsenic in foods is 50-100%. For calculations based on populations within each GEMS cluster, it is assumed that (i) roughly an equal number of men and women comprise each GEMS dietary cluster of nations; and (ii) that the individuals within each GEMS cluster consume roughly comparable amounts of the foodstuffs that are presented in the GEMS database, including across age groups and genders.

2.3 RISK CHARACTERIZATION

Toxicity values viz, reference doses and slope factors are used in this risk characterization step for estimating the likelihood of adverse effects occurring in populations exposed at different exposure levels. Studies based on occupational cohorts or studies in experimental animals typically provide data useful in this analysis (NRC, 1983).

In order to characterize the risk of bladder, lung and skin cancer due to foodborne arsenic, the data from dose–response and exposure assessment were integrated to quantify the burden of arsenic related cancers across the world. For each cancer type the respective slope factor was multiplied with the estimated range of daily dietary inorganic arsenic exposure and the population size of the individual GEMS cluster to obtain an annual gender-specific estimate of the additional number of foodborne arsenic related cancers.

To characterize the risk of coronary heart disease, a reference dose (RfD) was calculated based on the estimated benchmark dose lower bound (BMDL) values. Uncertainty factors were used to adjust a NOAEL, LOAEL, or benchmark dose in order to derive a RfD (USEPA BMD Technical Guidance, 2012). Uncertainty can be defined as "a lack of precise knowledge as to what the truth is, whether qualitative or quantitative" (NRC, 1994). Uncertainty differs from variability in that it can generally be reduced by further research. Uncertainty factors are applied as needed to account for extrapolation of results in experimental animals to humans, inter-individual variability including sensitive subgroups, extrapolation from a LOAEL to a NOAEL, extrapolation of results from subchronic exposures to chronic exposures, and database inadequacies.

3.0 LITERATURE REVIEW

3.1 ARSENIC INDUCED CANCERS

Epidemiological evidence supporting a causal role for arsenic in cancers was estimated as the first step to determine if there was substantial proof for bladder, lung and skin cancer in humans caused by arsenic. PubMed was searched for all epidemiology studies on arsenic in drinking water and cancer of bladder, lung and skin published from January 1966 through December 2012. Literature was also collected for studies on arsenic-induced renal cancer, prostate cancer and liver cancers. The following search terms were included: arsenic, arsenicals, water, cancer, lung, bladder, skin, liver, prostate, renal (kidney) and epidemiology. Studies that assessed the relation between arsenic exposure (determined using environmental measure of drinking water, biomarkers, or indirect measures (living in arsenic-endemic areas)) and clinical outcomes of disease endpoints were identified.

The exclusion criteria were: (i) Non- human studies (experimental studies); (ii) case reports and case series; (iii) no chronic arsenic exposure levels in general population settings (e.g., acute arsenic poisoning, use of arsenic trioxide as a chemotherapeutical agent, or lewisite, occupational exposure); (iv) studies that assess arsenic exposure through air; and (vi) studies originally published in another language besides English.

There is a significant amount of research literature from studies conducted in five major regions of the world with especially elevated levels of naturally occurring arsenic: southwestern and northeastern Taiwan, northern Chile, Cordoba Province in Argentina, Bangladesh, West Bengal (India) and other regions in the Ganga plain. These provide the strongest evidence for the association of human cancer with arsenic in drinking water (IARC, 2012).

A recent IARC report on several different Group 1 human carcinogens (IARC, 2012) indicated that the general population is primarily exposed to arsenic through the oral intake of contaminated food or water. Although inhalation of arsenic constitutes an occupational hazard for industrial workers, it is considered to be a minor exposure route for the general population (IARC, 2012).

This dissertation focuses on the epidemiological evidence based on cancer risk associated with the ingestion - rather than inhalation - of arsenic. As per IARC (2012), ecological studies are adequate to deduce causal inference for carcinogenic effects of arsenic in drinking water, mainly due to a large contrast in the exposure of different population subsets. Moreover, high ecological estimates of relative risk rule out the possibility of potential confounding with known causal factors (IARC, 2012).

3.1.1 Arsenic-induced bladder cancer

Multiple ecological, case-control and cohort studies indicate an association between arsenic exposure and bladder cancer (IARC, 2012). Ecological studies in southwestern and northeastern Taiwan have observed an increase in mortality from urinary bladder cancer due to exposure to arsenic via drinking water (Chen et al., 1985, 1988a; Wu et al., 1989, Chen et al., 1990). Tsai et

al. (1999) reported an increase in standardized mortality rate (SMR) values by gender during the period 1971 to 1994 as compared to the local and national reference groups. Comparable risks for bladder cancer were found by an additional study based in the blackfoot disease endemic areas of Taiwan (Chiang et al., 1993) using incidence records. Rivara et al. (1997) and Smith et al. (1998) found considerably elevated SMR values for bladder cancer in the periods 1950 to 1992 and 1989 to 1993 respectively, in the aforementioned high-risk region of Chile. Estimates of exposure to arsenic in drinking water were also reported to be associated with elevated SMRs in ecological studies in Cordoba province, Argentina (Hopenhayn-Rich et al., 1996, 1998).

Chen et al. (1986) reported an increasing trend in age-sex- adjusted odds ratios (OR) with an increase in the duration of consumption of artesian well-water containing arsenic. This was done in a case-control study conducted in the blackfoot disease endemic area (BFDEA) in Taiwan using death certificates.After adjusting for smoking and other factors from next-of-kin interviews, the highest risks were observed in populations exposed for over 40 years to arsenic containing well water, with an OR of 4.1 ($P<0.01$) in a multivariate analysis. The age-sex-adjusted OR of developing bladder cancer for those who had used artesian well water for 40 or more years was 3.90 ($P<0.01$) as compared with those who had never used artesian well water (Chen et al., 1986).

Numerous case–control studies that included an analysis of incident bladder cancer in urine samples, have observed a higher risk associated with arsenic among persons with higher MMA(V):DMA(V) ratios (Chen et al., 2003, 2005a), or alternatively, with a higher percentage of MMA(V) (Steinmaus et al., 2006; Pu et al., 2007a; Huang et al., 2008; IARC, 2012). Chen et al. (2003) reported a substantially increased risk of bladder cancer, in subjects with low

secondary arsenic methylation index, especially when combined with high cumulative arsenic exposure levels. Steinmaus et al. (2003) reported significantly elevated odds ratios for bladder cancer in smokers with arsenic intakes of >80 μg/day (highest 1-year average), 40 or more years prior to diagnosis (OR = 3.67; 95% CI:1.43–9.42), but not for ever and never smokers combined (OR = 1.78; 95% CI: 0.89–3.56) or for never smokers (OR = 0.31; 95% CI: 0.06–1.66). Among smokers, Karagas et al. (2004) reported an elevated OR for bladder cancer for the uppermost category of arsenic in toenails (OR 2.17, 95% CI: 0.92–5.11, for greater than 0.330 μg/g compared to less than 0.06 μg/g) in New Hampshire, US. These data suggested that ingestion of low to moderate arsenic levels may affect bladder cancer incidence and that cigarette smoking may act as a co-carcinogen.

Elevated bladder cancer risk was observed following chronic exposure to arsenic in multiple cohort studies in different parts of the world viz. southwestern and northeastern Taiwan (Chen et al., 1988b; Chiou et al., 1995, 2001), United Kingdom (Cuzick et al., 1992) and Japan (Tsuda et al., 1995). Chiou et al. (2001) reported an OR of 1.96 (95% CI: 0.94-3.61, based on 10 cases) in Taiwan. Morales et al. (2000) found estimated concentrations of arsenic in well water that were associated with a 1% increase in the risk of developing bladder cancer in a Taiwanese population. Aballay et al. (2012) reported an increased risk of bladder cancer in both sexes associated with arsenic exposure, based on age-standardized incidence rates for cancer in Argentina. In the BFDEA of Taiwan, Su et al. (2011) reported a decline in the incidence of bladder cancer with a reduction in arsenic intake from water.

Gibb et al. (2011) suggested that the ability of an epidemiologic study of arsenic exposure to detect associations with lung or bladder cancer could be impacted by the number of smokers in

the study population. Indeed, an increased risk of bladder cancer was reported only in smokers, exposed to relatively low concentrations of arsenic in drinking water in multiple studies (Bates et al., 2004; Karagas et al., 2004; Steinmaus et al., 2003; Gibb et al., 2011). Earlier studies in Finland and USA (Kurttio et al.,1999; Bates et al., 1995) also present evidence of an interaction between smoking and arsenic with respect to bladder cancer. In a meta-analysis of 10 studies, Begum et al. (2012) reported a strong correlation of the absolute risk of bladder cancer from ingested arsenic with smoking rates.

In a cohort study in Denmark, Baastrup et al. (2008) found no association between the time-weighted average/cumulative exposures and lung/bladder cancer in the incidence rate ratios (IRRs), adjusted for a variety of different variables. Relative risks were not reported by sex. Chen et al. (2010b) reported a significant monotonic increasing risk of urinary cancer ($p < 0.001$) with arsenic concentration in a cohort study in northeastern Taiwan. Compared with those consuming <10 μg arsenic/L, the age and sex-adjusted RRs (95% CIs) for arsenic levels 10–49.9, 50–99.9, 100–299.9, and ≥ 300 μg/L were 1.7 (0.56–5.19), 2.49 (0.73–8.59), 4.18 (1.3–12.8), and 7.73 (2.69–22.3), respectively. Urinary cancer RRs (95% CIs) for cumulative arsenic exposures 400–1,000, 1,000–5,000, 5,000–10,000, and ≥ 10,000 μg/L-year were 1.16 (0.29–4.64), 2.44 (0.91–6.5), 3.88 (1.18–12.7), and 7.55 (2.79–20.4), respectively, compared with < 400 μg/L-year.

3.1.2 Arsenic-induced lung cancer

A large body of ecological studies indicates that arsenic exposure is associated with increased risk for lung cancer mortality (IARC, 2012). In an ecological study based in the aforementioned

high-risk region of Chile, Rivara et al. (1997) found the standard mortality ratio to be increased for both sexes when compared to the national rates (men, SMR = 3.8 (95% CI, 3.5-4.1); women, SMR = 3.1 (95% CI, 2.7-3.7)). In a 50-year study of lung cancer mortality, the peak lung cancer mortality RRs were 3.61 (95% CI, 3.13- 4.16) for men and 3.26 (95% CI, 2.50- 4.23) for women in this region, compared within a low-exposure area in Chile (Marshall et al., 2007). An ecological study conducted in Cordoba, Argentina found increasing trends for lung cancer mortality with arsenic exposure. The SMR values in the high exposure groups were 1.77 for men and 2.16 for women, respectively (Hopenhayn-Rich et al., 1998).

Smith et al. (2006) compared mortality rates in Antofagasta, Chile in the period 1989-2000 with those in the rest of Chile. They found that the SMR values for lung cancer for the birth cohort born just before the high-exposure period (1950-1957) and exposed in early childhood was 7.0 (95% CI, 5.4-8.9; $p<0.001$). For those born during the high-exposure period (1958-1970) with probable exposure in-utero and early childhood, the SMR was 6.1 (95% CI, 3.5-9.9; $p<0.001$) for lung cancer (Smith et al., 2006). Based upon a meta-analysis, Begum et al. (2012) estimate about 4.51 additional lung cancer cases per 100,000 people for a maximum contamination level of 10μg/L of arsenic. In a case-control study in northern Chile, Ferreccio et al. (2000) found increasing levels of arsenic exposure during the years 1958-1970 to be associated with increased lung cancer risks (OR 7.1, 95% CI, 3.14-14.8).

Cohort studies in southwestern Taiwan observed a positive dose response relationship between the exposure to artesian well water and lung cancer. Morales et al. (2000) found estimated concentrations of arsenic in well water that were associated with a 1% increase in the risk of developing lung cancer in a Taiwanese population. The age-adjusted OR of developing lung

cancer was 3.39 for those who were exposed to arsenic in artesian well water for 40 or more years, as compared to those who had never used artesian well water (Chen et al., 1986). Chiou et al. (1995) reported a smoking-adjusted increased risk for lung cancer in relation to increasing cumulative exposure to arsenic.

Chen et al. (2004), Ferreccio et al. (2000) and Mostafa et al. (2008) suggested an interaction between smoking and arsenic with respect to lung cancer, as was evident by the significantly elevated RRs among smokers compared with nonsmokers, both groups being exposed to arsenic. In a cohort study, Chen et al. (2004) described 2,503 residents of southwestern Taiwan and 8,088 residents of northeastern Taiwan, who consumed arsenic-contaminated water for > 50 years. When compared with the referent group of <10 μg/L, the adjusted RRs (95% CIs) for lung cancers were 1.09 (0.63–1.91); 2.28 (1.22–4.27); 3.03 (1.62–5.69); and 3.29 (1.60–6.78) for average arsenic concentrations of 10–99, 100–299, 300–699, and ≥ 700 μg/L, respectively. The trend was statistically significant and strong synergism was reported between arsenic in water and cigarette consumption. For those smoking ≥ 25 pack-years, RRs (95% CIs) for lung cancer were 3.8 (1.29–11.2), 5.93 (2.19–16.1), and 11.10 (3.32–37.2) for average arsenic concentrations of < 10, 10–699, and ≥ 700 μg/L, respectively.

In the northeastern Taiwan cohort, Chen et al. (2010a) studied 8,086 residents. The RRs and 95% CIs for 100–300 and >300 μg arsenic/L when compared with <10 μg arsenic/L were 1.54 (0.97–2.46) and 2.25 (1.43–3.55), respectively. Although no apparent increased risk was reported at concentrations between 10 and 100 μg/L arsenic, these associations tended to increase with longer durations of exposure. A synergistic effect of arsenic exposure and cigarette smoking was found for squamous- and small-cell carcinomas, but not for adenocarcinoma. A drastic drop in

the lung cancer incidence of BFDEA was reported by Su et al. (2011) as a positive outcome of municipal water supply in Taiwan.

3.1.3 Arsenic-induced non-melanoma skin cancer

An extensive body of literature definitively links the ingestion of arsenic to increased incidence of skin cancer. Multiple ecological studies based on mortality from skin cancer in Taiwan found consistent gradients of increasing risk with average level of arsenic in drinking water, as measured on the precinct or township level (Chen et al., 1985, 1988a; Chen et al., 1990; Wu et al., 1989; Tsai et al., 1999; IARC, 2012).

SMR value of 3.2 (95% CI: 2.1–4.8) was observed for skin cancer mortality in one region of Chile with high arsenic exposures in drinking water during 1976-92, as compared to a relatively unexposed control region in Chile (Rivara et al., 1997). Elevated SMR values were reported in both sexes (7.7, 95% CI: 4.7–11.9 among men and 3.2, 95% CI: 1.3–6.6 among women) for the years 1989-1993 in a later study in this high-risk region using the national mortality rates as reference (Smith et al., 1998).

An eight-fold difference was observed in the prevalence of skin cancer lesions from the highest to the lowest category of arsenic concentration (>600 μg/L and <300 μg/L, respectively) in artesian wells in southwestern Taiwan, based on a survey in 37 villages with 40,421 participants (Tseng et al., 1968). Chen et al. (1988b) reported an SMR of 28 (95% CI: 11–59) for skin cancer deaths in a retrospective cohort study of 789 blackfoot disease patients in Taiwan (based on seven observed deaths), using Taiwan regional rates as reference. In another cohort study (654 participants) in southwestern Taiwan, Hsueh et al., (1995) observed an incidence rate of 14.7

cases of skin cancer/1000 person–years. They found that the risks were significantly related to the duration of consumption of artesian well-water, average concentration of arsenic, duration of living in the area endemic for blackfoot disease and the cumulative arsenic exposure index. Hsueh et al. (1995) reported similar findings in a nested case-control study conducted within the same cohort. Additionally, case-control studies in New Hampshire, USA suggested increased risk of skin cancer was associated with toenail arsenic, especially among smokers (Karagas et al., 2001, 2004; Hughes et al., 2011). In a case–control study, conducted in areas of Hungary, Romania, and Slovakia, Leonardi et al. (2012) observed a positive association between BCC and exposure to inorganic arsenic through drinking water with concentrations < 100 μg/L. They reported an adjusted OR of 1.18 (95% CI: 1.08, 1.28) per 10μg/L increase in average lifetime water inorganic arsenic concentration.

In a prospective study based on individual-level data from Bangladesh, Melkonian et al. (2011) observed significant synergistic effects between measures of arsenic exposure, smoking and fertilizer use. Relative excess risks for the interactions between smoking status and arsenic exposure were 0.12 (95% CI: 0.06, 0.19) for water arsenic and 0.11 (95% CI: 0.05, 0.15) for urinary arsenic measures, respectively. These relative risk values support a role for smoking in modification of the effect of long-term arsenic exposure on skin lesions that are considered precursors to arsenic-related skin cancer. IARC (2012) notes that the histological types of skin cancer were not reported in a majority of the studies. Moreover, because of the notably high SMR values, it is unlikely that the observed associations are entirely due to confounding factors such as access to health care.

3.1.4 Other cancers

Liver Cancer

Numerous studies from southwestern Taiwan have evaluated the risk of liver cancer due to arsenic exposure via drinking water (Chen et al., 1985, 1988a; Wu et al., 1989; Chen et al., 1990; Chiang et al., 1993; Tsai et al., 1999; IARC, 2012). Rivara et al. (1997) reported a relative risk of 1.2 (95% CI: 0.99–1.6) in arsenic-exposed region II compared with region VIII, Chile. Liver cancer among children born in region II during 1950- 1957 and those exposed in-utero was found to have a RR of 10.6 (95% CI: 2.9–39.3, $P < 0.001$) as compared to the rates in region V, Chile (Liaw et al., 2008). Smith et al. (1998) found that the liver cancer mortality in region II, Chile during the period 1989–93 among persons ≥ 30 years of age was not significantly elevated, using national rates as reference, with the SMR values for both sexes being 1.1 (95% CI: 0.8–1.5). However, Hopenhayn-Rich et al. (1998) did not find the SMR values to be related to arsenic exposure in Cordoba Province, Argentina. Morales et al. (2000) find increased liver cancer risk in more highly arsenic-exposed Taiwanese populations, but did not account for confounding factors such as aflatoxin exposure or chronic infection with hepatitis B virus (HBV).

As noted in IARC (2012), *"the finding of an association with liver cancer in Taiwan but not in South America may reflect a more sensitive population in the former region, due to endemic hepatitis B. The elevated risk of those exposed in-utero and as young children may reflect a combination of greater biological vulnerability in early life (Waalkes et al., 2007) plus the fact that young children consume 5–7 times more water per kilogram body weight per day than adults (NRC, 1993)."*

This dissertation relied on the hazard identification of IARC (2012) that did not find sufficient evidence to associate arsenic in drinking water with liver cancer. Recent studies particularly from countries, where exposures to aflatoxins and Hepatitis B as well as the background rate of liver cancers is not as high as in Taiwan, further support an association between arsenic exposure and liver cancer. Smith et al. (2012) have reported an increased risk of liver cancer following in utero and childhood exposure to arsenic in drinking water in Chile.

Renal Cancer

A vast body of literature reports the relation between arsenic exposure via drinking water and cancers of the bladder and kidney (Chen et al., 1985, 1988a; Wu et al., 1989; Chen et al., 1990; Tsai et al., 1999; IARC, 2012). However, as noted by the IARC Working Group (2012), "*kidney cancers consist of both renal cell carcinoma and transitional cell carcinoma of the renal pelvis, the latter often being of the same etiology as bladder cancer."* Because arsenic causes transitional cell carcinoma of the bladder the possibility of the risk estimate for total kidney cancer to be diluted cannot be denied.

Prostate Cancer

A recent NRC report (NRC, 2013) acknowledges modest epidemiologic evidence of an association between arsenic and prostate cancer. Arsenic exposure has been linked to prostate cancer in ecological studies. The studies conducted in Taiwan reported a significant dose-response relationship between the level of arsenic in drinking water and the risk for prostate cancer mortality using several methodological approaches and comparison populations, direct

and indirect standardization of rates (Chen et al., 1985, 1988a; Wu et al., 1989; Chen et al., 1990; Tsai et al., 1999; IARC, 2012).

Arsenic concentrations in well water were found to be associated with malignant neoplasms of prostate cancer resulting in an age-adjusted increase in mortality (Chen et al., 1990). Causality of arsenic and prostate cancer are also supported by an observed decline in mortality with arsenic levels in water (Yang et al., 2008). An SMR of 1.45 supporting a dose–response relationship between arsenic in drinking water and prostate cancer was observed in an ecologic analysis of a cohort of Mormon residents of Utah (Lewis et al.,1999). Garcia-Esquinas et al (2013) in the Strong Heart Study of an American Indian cohort in US states of Arizona, North Dakota and South Dakota report an association between baseline urinary arsenic concentrations and increased prostate-cancer mortality.

Nonetheless, contradictory observations have also been made in other epidemiological studies, regarding associations between prostate cancer and arsenic. Based on mortality data in two regions of Chile with high arsenic levels in water, Rivara et al. (1997) report a risk ratio (RR) of 0.9. While in a cohort study in Denmark, Baastrup et al. (2008) did not observe any increase in prostate cancer mortality.

3.2 ARSENIC-INDUCED CARDIOVASCULAR DISEASE

As the first step to determine substantial epidemiological evidence supporting a causal role for arsenic in cardiovascular disease, a thorough literature search was conducted. PubMed was searched for all epidemiology studies on arsenic in drinking water and cardiovascular disease.

Existing evidence between arsenic exposure and cardiovascular disease was summarized in a recent revised systematic review and meta-analysis (Moon et al., 2012). This review was published as an update to an earlier systematic review (Navas-Acien et al., 2005) associating cardiovascular diseases with arsenic exposure. These reviews strengthen the evidence for a causal association between high chronic arsenic exposure and clinical cardiovascular endpoints, while recognizing a need for additional high quality studies at low to moderate arsenic levels.

For this research, hazard assessment relied on the literature collected in these two reviews as well as a search for studies that came out between June 2012 and July 2013.This period was selected to include the studies published after Moon et al. (2012) review that were relevant to this research. PubMed was searched for epidemiological studies investigating the relation of arsenic with cardiovascular disease with following free text and Medical Subject Headings: arsenic, arsenicals, arsenic poisoning, arsenite, arsenate, atherosclerosis, carotid artery diseases, coronary artery disease, cardiovascular diseases, myocardial infarction, stroke, cerebrovascular disorders, peripheral vascular diseases, peripheral arterial disease, mortality, atherosclerosis, arteriosclerosis, blackfoot disease.

Studies that assess the relation between arsenic exposure (determined using environmental measure of drinking water, biomarkers, or indirect measures) and clinical outcomes of disease endpoints were identified. The exclusion criteria were the same as those used for the literature review of arsenic induced bladder, lung and skin cancers (section 3.1):

(i) non- human studies; (ii) case report or case series; (iii) no chronic arsenic exposure levels in general population settings (e.g., acute arsenic poisoning, use of arsenic trioxide as a chemotherapeutical agent, or lewisite, occupational exposure); (iv) no original research (i.e.,

reviews, editorials, non-research letters); (v) no clinical cardiovascular outcomes (e.g., subclinical atherosclerosis); and (vi) studies originally published in another language besides English.

17 studies met the inclusion criterion. Of these, 6 studies were included in the systematic review by Moon et al. (2012) and 10 studies in that by Navas-Acien (2005). Only 1 study published after Moon et al. (2012), namely Moon et al. (2013) was included in this review (Tables 3, 4, 5, 6). 10 studies were conducted in high arsenic exposure areas of Taiwan, Bangladesh and Inner Mongolia, and 7 studies were conducted in low to moderate arsenic exposure areas in the US, Japan and Spain.

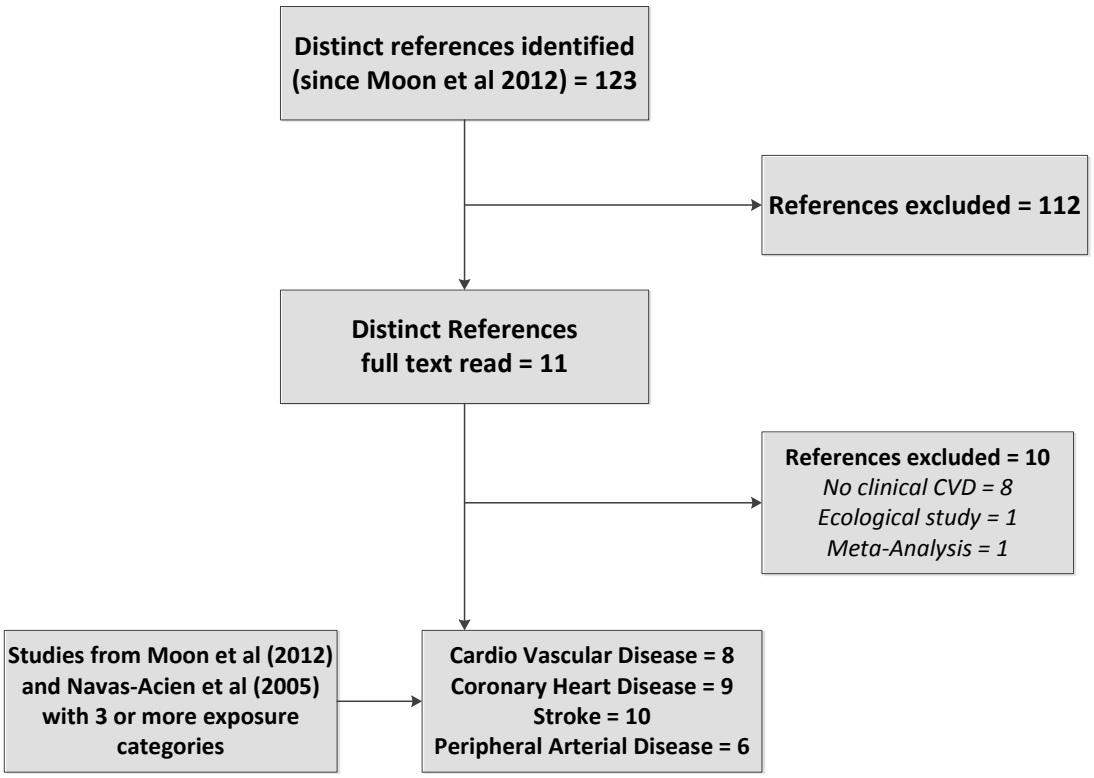


Figure 4 - Selection process used in a literature review of studies on the relation between arsenic and cardiovascular disease

All the studies can be broadly categorized into high exposure or low exposure analyses depending on the highest mean exposure levels of arsenic detected in the study region. The cut-off for high exposure studies was kept at more than or equal to 150 µg/L of arsenic. Of the 7 cohort studies, 4 were prospective cohort (Moon et al., 2013; Chen et al., 2011; Sohel et al., 2009; Chen et al., 1996) and 3 were retrospective cohort studies (Wade et al., 2009; Lewis et al.,

1999; Lisabeth et al., 2010). 4 studies were ecological (Medrano et al., 2010; Yoshikawa et al,, 2008; Engel et al., 1994; Wu et al., 1988). 4 studies were cross-sectional in design (Tseng et al., 1996, 2003 and 2005; Zierold et al., 2004; Chiou et al., 1997) and 1 was a case control study (Chen et al., 1988).

Studies differed in their assessment of arsenic exposure: while some used environmental measures, such as arsenic in drinking water at the region/municipal village level (Chen et al., 2011; Sohel et al., 2009; Medrano et al., 2010; Engel et al., 1994; Chen et al., 1996; Zierold et al., 2004; Wade et al., 2009; Lisabeth et al., 2010; Chen et al., 1988), at the household/individual level (Wade et al., 2009; Chiou et al., 1997), or in air (Yoshikawa et al., 2008); other studies analyzed exposure by calculating an arsenic exposure index accounting for duration of water consumption (Tseng et al., 2003; Lewis et al., 1999; Tseng et al., 1996; Tseng et al., 2005).

The studies also adopted varied methods of ascertainment of the different CVD outcomes. Studies ascertaining cardiovascular disease reported mortality by medical records and verbal autopsy (Chen et al., 2011; Sohel et al., 2009; Wade et al., 2009) as well as death certificates (Wu et al., 1989; Medrano et al., 2010; Engel et al., 1994) or a combination of these as well as hospitalization records (Moon et al., 2013) or relied on national health maps (Yoshikawa et al., 2008). Studies on coronary heart disease mostly used mortality endpoints (Chen et al., 2011; Chen et al., 1996; Wu et al., 1989; Medrano et al., 2010; Lewis et al., 1999; Engel et al., 1994), although 2 studies ascertained prevalent cases (Tseng et al., 2003; Zierold et al., 2004) and 1 study identified mortality, as well as total incident cases (Moon et al., 2013). In the studies reporting prevalence endpoints, coronary heart disease was assessed by self-report (Zierold et al., 2004) or by electrocardiogram (Tseng et al., 2003). Studies on stroke and peripheral arterial

diseases mostly reported mortality, although some focused only on disease prevalence. Stroke mortality was reported in high exposure studies (Chen et al., 2011; Wade et al., 2009; Wu et al., 1989) as well as studies conducted in areas with low to moderate exposure to arsenic (<150µg/L) (Moon et al., 2013; Medrano et al., 2010; Yoshikawa et al., 2008; Zierold et al., 2004; Lewis et al., 1999; Engel et al., 1994). One study from Japan ascertaining stroke and cardiovascular disease was identified to be unique as it described exposure to arsenic in air. This led to it being incomparable to the rest of the studies that focus on oral route of arsenic exposure in the general population. Moreover it cannot be denied that in this population from Japan, rice could potentially be a major source of arsenic exposure instead of air. In one study from United States, mortalities from vascular diseases were increased in counties where arsenic levels were > 20 ppb relative to those with < 10 ppb (Engel et al., 1994).

The association with high arsenic exposure and peripheral arterial disease (such as blackfoot disease) seems to be limited to populations that have poor nutrition (Tseng et al., 2005), while that between arsenic and cardiovascular disease is inconclusive at both low and high exposure levels. Of all the cardiovascular diseases associated with arsenic exposure, coronary heart disease has a well-established clinical cardiovascular endpoint that has been uniformly reported across different studies (Chen et al., 2011; Chen et al., 1996; Wu et al., 1989, Medrano et al., 2010; Lewis et al., 1999; Engel et al., 1994; Moon et al., 2013). Since the epidemiological evidence for an association between arsenic exposure and CHD is stronger than any other non-cancer disease (NRC, 2013), CHD incidence was considered the best disease endpoint for the purpose of deriving a benchmark dose for arsenic.

Table 3 - Studies of arsenic exposure and clinical outcome: Cardiovascular disease

Study Author & Year	Design	Population	Men (%)	Age Range (years)	Arsenic Exposure Assessment	Exposure Categories - RANGE	Exposure Categories - UNITS	Endpoint Ascertain--ment	Outcome	No. of Cases/ NonCases	RR type	Relative Risk	Lower 95% CI	Upper 95% CI	Adjustment Factors
HIGH EXPOSURE AREAS >150 µg/L															
Chen et al 2011	Prospective Cohort	Araihazar, Bangladesh	NR	18-75	Baseline urine, creatinine-adjusted	6.6-105.9	µg/g creatinine	Verbal autopsy, medical records	CVD mortality	44 deaths	HR	1	--	--	Age, sex, education, BMI, smoking status, and changes in arsenic concentration between visits
						106.0-199.0				48 deaths		1.15	0.77	1.72	
						199.1-351.8				54 deaths		1.56	1.03	2.38	
						351.9-1100				46 deaths		1.55	1.01	2.37	
					Baseline concentration of well arsenic	0.1-12	µg/L			43 deaths		1			Baseline age, sex, BMI, smoking status, educational attainment (years), and changes in arsenic concentration adjusted for urinary creatinine (µg per g of creatinine) between visits
						12-62.0				51 deaths		1.21	0.8	1.84	
						62.1-148.0				41 deaths		1.24	0.8	1.93	
						148.1-864				63 deaths		1.46	0.96	2.2	
Sohel et al 2009	Prospective Cohort	Matlab, Bangladesh	49.3	15- 75+	water sample	<10	µg/L	Verbal autopsy	CVD mortalit	129 deaths	HR	1	--	--	Age, sex, asset score, and

						10 to 49				153 deaths		1.03	0.82	1.29	
						50 to 149				476 deaths		1.16	0.96	1.4	
						150 to 299				388 deaths		1.23	1.01	1.51	
						300 +				152 deaths		1.37	1.07	1.77	
Wade et al 2009#	Retrospective Cohort	Shahai village, Inner Mongolia, China	50	0 -80+	Household, shared or community wells levels	0-5	µg/L	Verbal description by the family and medical records	CVD mortality	36 deaths	IRR	1	--	--	Age, sex education, smoking, alcohol use, farm work
						5.1-20				12 deaths		0.75	0.37	1.51	
						20.1-100				37 deaths		1.28	0.79	2.07	
						100.1-300				15 deaths		1.6	0.87	2.95	
						>300				2 deaths		5.08	1.45	17.81	
Wu et al 1989	Ecological	SW Taiwan	52	0 -80+	Village well water	<0.30	mg/L	Death certificate	CVD mortality	683 deaths	SMR	1	--	--	Age
						0.30 - 0.59				456 deaths		1.232	1.027	1.477	
						≥ 0.6				229 deaths		1.494	1.286	1.736	
LOW-MODERATE EXPOSURE AREAS <150 µg/L															
Moon et al 2013	Prospective cohort	USA (3 American Indian	39.8	45-74	Baseline urine arsenic levels µg/g	<5.8	µg/g creatinine	Hospitalization records, death	CVD incidence (fatal	265 fatal and non-fatal cases	HR	1	--	--	Sex, education, smoking status, body mass index (kg/m^2), and

						5.8-9.7				297		1.14	0.95	1.35	
						9.8-15.7				291		1.05	0.87	1.26	
						>15.7				331		1.32	1.05	1.28	
					Baseline urine arsenic levels µg/g creatinine	<5.8			CVD mortality	86 cases		1			
						5.8-9.7				95 cases		1.12	0.83	1.52	
						9.8-15.7				115 cases		1.26	0.92	1.73	
						>15.7				143 cases		1.65	1.2	2.27	
Medrano et al 2010	Ecological	Spain (651 municipalities)	NR	>20	Municipal drinking water levels	<1	µg/L	Death certificate	CVD mortality	146,567 deaths	SMR	1	--	--	Age, sex and provincial level variables including (income, hospital beds, CV risk factors, dietary factors and water characteristics)
						1-10				41,957 deaths		1.02	0.99	1.06	
						>10				11,852 deaths		1.03	0.98	1.08	
Yoshikawa	Ecological	Japan (264	49*	NR	5 year	<0.77	ng/m^3	National	CVD	14,247	SM	1	--	--	Age, sex

						0.77 to 0.9						0.977	0.942	1.013	
						0.9 to 1.04						1	0.963	1.039	
						1.04 to 1.2						1.014	0.975	1.055	
						1.2 to 1.36						0.961	0.906	1.02	
						1.36 to 1.60						1.005	0.958	1.055	
						1.6 to 1.77						1.001	0.966	1.038	
						1.77 to 2.2						1.051	1.016	1.086	
						2.2 to 2.7						0.958	0.918	1	
						>=2.7						1.029	0.995	1.064	
Engel and Smith 1994	Ecological	US (30 counties)	45.3	35 to >64	Drinking water level at county level	5 to 10	µg/L	Death certificate	CVD mortality	76,190 deaths	SMR	1	--	--	Age, sex
						10 to 20				24,037 deaths		0.8	0.788	0.812	
						>20				6,157 deaths		0.948	0.855	1.051	

Notes:
descriptive information abstracted from Xia 2009 but RR values from Wade 2009
^ Overall cases
* calculated from Table 1 of Yoshikawa et al. (2008)
NR = Not Reported; OR - Odds Ratio; SMR – Standard Mortality Rate; RR - Relative Risk; HR – Hazard ratio

Table 4 - Studies of arsenic exposure and clinical outcome: Coronary heart disease

Study Author & Year	Design	Population	Men (%)	Age Range (years)	Arsenic Exposure Assessment	Exposure Categories - RANGE	Exposure Categories - UNITS	Endpoint Ascertain-ment	Outcome	No. of Cases/ NonCases	RR type	Relative Risk	Lower 95% CI	Upper 95 % CI	Adjustment Factors
HIGH EXPOSURE AREAS >150 µg/L															
Chen et al 2011	Prospective Cohort	Araihazar, Bangladesh	NR	18-75	Baseline urine, creatinine-adjusted	6.6-105.9	µg/g creatinine	Verbal autopsy, medical records	CHD mortality	17 deaths	HR	1	--	--	Age, sex, education, BMI, smoking status, and changes in arsenic concentration between visits
						106.0-199.0				18 deaths		1.29	0.66	2.51	
						199.1-351.8				17 deaths		1.47	0.72	3.01	
						351.9-1100				17 deaths		1.9	0.91	3.98	
					Baseline concentration of well arsenic	0.1-12	µg/L			14 deaths		1	--	--	Baseline age, sex, BMI, smoking status, educational attainment (years), and changes in arsenic concentration adjusted for urinary creatinine (µg per g of creatinine) between visits
						12-62.0				16 deaths		1.22	0.56	2.65	
						62.1-148.0				15 deaths		1.49	0.79	3.19	
						148.1-864				26 deaths		1.94	0.99	3.84	
Tseng et al 2003	Cross-sectional	SW Taiwan	44	30 to >=60	CEI from village	0	mg/L-y	Electrocardiogram	CHD prevalenc	4 cases/73 non-cases	OR	1	--	--	Age, sex, smoking, BMI,

						0.1 to 14.9				19 cases/159 non-cases		1.6	0.48	5.34	
						>=15				50 cases/157 non cases		3.6	1.11	11.65	
Chen et al 1996	Prospective Cohort	SW Taiwan	52	40 to >=70	CEI from community drinking water	0	mg/L-y	Death certificates	CHD mortality	4 deaths/505 non-cases	RR	1	--	--	Age, sex, smoking, BMI, lipids, hypertension, diabetes mellitus
						0.1-9.9				3 deaths/349 non-cases		2.16	0.46	10.16	
						10.0-19.9				5 deaths/465 non-cases		3.33	0.83	13.45	
						>20				13 deaths/420 non-cases		4.9	1.36	17.68	
Wu et al 1989	Ecological	SW Taiwan	52	0 to >80	village drinking	<0.30	ppm	Death certificate	CHD mortality	232 deaths	SMR	1	--	--	Age, sex

						0.30 to 0.59				178 deaths		1.432	1.05	1.953	
						>= 0.6				99 deaths		1.854	1.441	2.38	
LOW-MODERATE EXPOSURE AREAS <150 µg/L															
Moon et al 2013	Prospective cohort	USA (3 American Indian Communities)	39.8	45-74	Baseline urine arsenic levels µg/g creatinine	<5.8	µg/g creatinine	Hospitalization records, death records and clinic visits	CHD incidence (fatal and non-fatal)	202 fatal and non-fatal cases	HR	1	--	--	Sex, education, smoking status, body mass index (kg/m^2), and low-density lipoprotein cholesterol level
						5.8-9.7				206 fatal and non-fatal cases		1.05	0.86	1.28	
						9.8-15.7				197 fatal and non-fatal cases		0.95	0.77	1.19	
						>15.7				241 fatal and non-fatal cases		1.3	1.04	1.62	
					Baseline urine arsenic levels µg/g creatinine	<5.8			CHD mortality	68 cases		1	--	--	
						5.8-9.7				67 cases		0.99	0.7	1.41	
						9.8-15.7				87 cases		1.18	0.83	1.69	
						>15.7				119 cases		1.71	0.19	1.4	
Medrano et al 2010	Ecological	Spain (651 municipalit	NR	>20	Municipal drinking	<1	µg/L	Death certificate	CHD mortality	46,182 deaths	SMR	1	--	--	Age, sex and provincial level

						1 to 10				13,213 deaths		1.05	1.01	1.1	
						>10				4,062 deaths		1.02	0.96	1.08	
Zierold et al 2004	Cross-sectional	USA (survey participants with private wells)	NR	Mean = 62	Drinking water level	<2	µg/L	Self-report	CHD prevalence	128 cases/1,057 non cases^	OR	1	--	--	Age, sex, smoking, BMI
						>2-<10						1.52	1	2.35	
						>10 (up to 2389)						1.54	0.9	2.68	
Lewis et al 1999	Retrospective Cohort (Analyzed as Ecological)	USA (Mormons)	52	>1	CEI from community drinking water	14 to 166	µg/L	Death certificates	CHD mortality	411 cases/3,647 non-cases^	SMR	1	--	--	Age, sex
												0.896	0.715	1.122	
												0.863	0.676	1.102	
Engel and Smith 1994	Ecological	USA (30 counties)	49.9	0 to >=65	county drinking water level	5 to 10	µg/L	Death certificates	CHD mortality	47,090 deaths	SMR	1	--	--	Age, sex
						10 to 20				13299 deaths		0.739	0.666	0.819	
						>20				3442 deaths		0.842	0.76	0.934	

Notes:
^ Overall cases and non-cases; NR - Not Reported; CEI - Cumulative Exposure Index; OR - Odds Ratio; SMR – Standard Mortality Rate; RR - Relative Risk; HR – Hazard ratio

Table 5 - Studies of arsenic exposure and clinical outcome: Stroke

Study Author & Year	Design	Population	Men (%)	Age Range (years)	Arsenic Exposure Assessment	Exposure Categories - RANGE	Exposure Categories - UNITS	Endpoint Ascertain--ment	Outcome	No. of Cases/ NonCases	RR type	Relative Risk	Lower 95% CI	Upper 95% CI	Adjustment Factors
HIGH EXPOSURE AREAS >150 µg/L															
Chen et al 2011	Prospective Cohort	Araihazar, Bangladesh	NR	18-75	Baseline urine, creatinine-adjusted	6.6-105.9	µg/g creatinine	Verbal autopsy, medical records	Stroke mortality	20 deaths	HR	1	--	--	Age, sex, education, BMI, smoking status, and changes in arsenic concentration between visits
						106.0-199.0				20 deaths		0.96	0.52	1.79	
						199.1-351.8				27 deaths		1.6	0.88	2.9	
						351.9-1100				15 deaths		1.03	0.53	2.03	
					Baseline concentration of well arsenic	0.1-12	µg/L			19 deaths		1	--	--	Baseline age (years), sex, BMI, smoking status, educational attainment (years), and changes in arsenic concentration adjusted for urinary creatinine (µg per g of creatinine) between visits
						12-62.0				26 deaths		1.35	0.75	2.43	
						62.1-148.0				18 deaths		1.2	0.63	2.27	
						148.1-864				22 deaths		1.07	0.54	2.12	
Wade et al	Retrospe	Shahai	50	0 to	Household,	0-5	µg/L	Verbal	Stroke	40 deaths	IRR	ref.	--	--	Age, sex

						5.1-20				13 deaths		0.62	0.33	1.18	
						20.1-100				20 deaths		0.65	0.38	1.12	
						100.1-300				6 deaths		0.58	0.26	1.29	
						>300				1 deaths		1.64	0.31	8.77	
Chiou et al 1997	Cross-Sectional	Northeast Taiwan	50	40 to >= 70	Household drinking water	<0.1	µg/L	Self report and medical records	Stroke prevalence	9 cases/995 non-cases	OR	1	--	--	Age, sex, smoking, alcohol,

						0.1-50				65 cases/3371 non cases		2.53	1.47	4.35	
						50.1-299.9				38 cases 1790 non-cases		2.78	1.55	4.97	
						≥300				19 cases/679 non-cases		3.6	1.83	7.11	
Wu et al 1989	Ecological	Taiwan	52	All ages	village drinking water level	<0.30	ppm	Death certificates	Stroke mortality	243 deaths	SMR	1	--	--	Age, sex
						0.30 to 0.59				141 deaths		1.058	0.86	1.302	
						>= 0.6				74 deaths		1.288	0.993	1.671	
LOW-MODERATE EXPOSURE AREAS <150 µg/L															
Moon et al 2013	Prospective cohort	USA (3 American Indian Communities)	39.8	45-74	Baseline urine arsenic levels µg/g creatinine	<5.8	µg/g creatinine	Hospitalization records, death records and clinic visits	Stroke incidence (fatal and non-fatal)	55 fatal and non-fatal cases	HR	1	--	--	Sex, education, smoking status, body mass index (kg/m^2), and low-density lipoprotein cholesterol level
						5.8-9.7				75		1.18	0.82	1.69	
						9.8-15.7				62		1.16	0.77	1.72	
						>15.7				72		1.47	0.97	2.21	
					Baseline urine arsenic levels µg/g creatinine	<5.8			Stroke mortality	6 cases		1	--	--	
						5.8-9.7				17 cases		1.41	0.54	3.67	
						9.8-15.7				13 cases		2.16	0.77	6.09	
						>15.7				18 cases		3.03	1.08	8.5	
Lisabeth et al 2010	Retrospective	US (1 county in	47	>=45	Average zip code	0.3 - <4.5	µg/L	Hospital database	Stroke hospita	14,033 admission	RR	1	--	--	Age, sex, income, race

						4.5 - <7.8						1.264	0.741	2.155	
						7.8 - <9.4						2.398	1.67	3.45	
						9.4 - <19.0						1.38	0.717	2.667	
						19 - ≤22.3						2.743	1.67	4.525	
Medrano et al 2010	Ecological	Spain (651 municipalities)	NR	>20	Municipal drinking water levels	<1	µg/L	Death certificates	Stroke mortality	38953 deaths	SMR	1	--	--	Age, sex and provincial level variables including income, hospital beds, cardiovascular risk factors, dietary factors and water characteristics
						1 to 10				11862 deaths		1	0.96	1.05	
						>10				3211 deaths		1.02	0.95	1.09	
Yoshikaw	Ecologic	Japan (264	47	NR	5 year	<0.77	ng/m^3	National	Stroke	13,596	SM	Ref	--	--	Age, sex

						0.77 to 0.9						1.007	0.97	1.044	
						0.9 to 1.04						1.014	0.98	1.055	
						1.04 to 1.2						1.07	1.028	1.112	
						1.2 to 1.36						0.982	0.95	1.02	
						1.36 to 1.60						0.94	0.903	0.98	
						1.6 to 1.77						0.97	0.94	1.01	
						1.77 to 2.2						0.95	0.92	0.98	
						2.2 to 2.7						0.95	0.92	0.99	
						>=2.7						1.015	0.98	1.05	
Zierold et al 2004	Cross-sectional	USA (survey participants with private wells)	NR	Mean= 62	Drinking water level	<2	µg/L	Self-report	Stroke prevalence	31 cases/ 1154 non-cases^	OR	1	--	--	Age, sex, smoking, BMI
						>2-<10						0.93	0.4	2.14	
						>10 (up to 2389)						1.53	0.6	4.07	
Lewis et al	Retrospe	USA	52	>1	Cumulative	14 to	µg/L	Death	Stroke	176 cases/	SM	1	--	--	Age, sex

												0.792	0.424	1.482	
												0.685	0.474	0.99	
Engel and Smith 1994	Ecological	USA (30 counties)	49.9	0 to >=65	county drinking water level	5 to 10	µg/L	Death certificates	Stroke mortality	15,166 deaths	SMR	1	--	--	Age, sex
						10 to 20				4,628 deaths		0.78	0.69	0.87	
						>20				1,169 deaths		0.91	0.74	1.1	

Notes:
descriptive information abstracted from Xia 2009 but RR values from Wade 2009; OR - Odds Ratio; SMR – Standard Mortality Rate; RR - Relative Risk; HR – Hazard ratio
^ Overall cases and non-cases
* Overall admissions for all exposure categories

Table 6 - Studies of arsenic exposure and clinical outcome: Peripheral Arterial disease

Study Author & Year	Design	Population	Men (%)	Age Range (years)	Arsenic Exposure Assessment	Exposure Categories - RANGE	Exposure Categories - UNITS	Endpoint Ascertain--ment	Outcome	No. of Cases/ NonCases	RR type	Relative Risk	Lower 95% CI	Upper 95% CI	Adjustment Factors
HIGH EXPOSURE AREAS >150µg/L															
Tseng et al 2005	Cross-sectional	SW Taiwan	46	≥ 30	total As in urine; CEI in well water	≤64.33 or >64.33	µg/L and mg/L-y	Ankle brachial index	PAD prevalence	54 cases/425 non-cases*	OR	1	--	--	Age, sex, BMI, cholesterol, alcohol consumption
						≤64.33						3.34	0.6	12.8	
						>64.33						3.84	0.86	17.25	
Tseng et al 1996	Cross-sectional	Taiwan	45	53 (mean)	Cumulative exposure index from village well water	0	mg/L-years	Ankle-brachial index	PAD prevalence	69 cases/513 non-cases*	OR	1	--	--	Age, sex, smoking, BMI, lipids, hypertension, diabetes mellitus
						0.1 to 19.9						2.77	0.84	9.14	
						>20						4.28	1.26	14.54	
Wu et al 1989	Ecological	SW Taiwan	35	All ages	village drinking water level	<0.30	mg/L	Death certificates	PAD mortality	42 deaths	SMR	1	--	--	Age, sex
						0.30 to 0.59				58 deaths		2.601	1.75	3.87	
						≥0.6				22 deaths		2.363	1.4	3.98	
Chen	Case	Taiwan	49	<50 -	Years of	0	years	Clinical	BFD	241	OR	1	--	--	Age, sex, diet,

						1 to 29						3.04	1.58	5.86	
						≥30						3.47	1.66	7.23	
LOW-MODERATE EXPOSURE AREAS <150 µg/L															
Lewis et al 1999	Retrospective Cohort (Analyzed as Ecological)	USA (Mormons)	52	>1	Cumulative exposure index from Community drinking water	14 to 166	µg/L	Death certificates	PAD mortality	47 cases/4011 non-cases	SMR	1	--	--	Age, sex
												0.842	0.437	1.624	
												0.609	0.283	1.311	
Engel and Smith 1994	Ecological	USA (30 counties)	43	0 - ≥65	County drinking water level	5 to 10	µg/L	Death certificates	PAD mortality	4,823 deaths	SMR	1	--	--	Age, sex
						10 to 20				1,759 deaths		1	0.947	1.056	
						>20				621 deaths		1.58	1.34	1.88	

Notes:
*Overall cases and non-cases
CEI - Cumulative Exposure Index; OR - Odds Ratio
PAD - Peripheral arterial disease; SMR – Standard Mortality Rate
BFD - Blackfoot disease; RR - Relative Risk
BMI - Body Mass Index; HR – Hazard ratio

4.0 GLOBAL BURDEN OF DISEASE FOR SKIN, LUNG AND BLADDER CANCER CAUSED BY ARSENIC IN FOOD

4.1 ABSTRACT

Arsenic is a naturally occurring metalloid that poses a significant human cancer risk. While water consumption provides the majority of human exposure, millions of individuals worldwide are significantly exposed to arsenic through naturally occurring levels of arsenic in grains, vegetables, meat and fish, as well as through food processed with water containing arsenic. This dissertation estimated the global burdens of disease for bladder, lung and skin cancers attributable to inorganic arsenic in food. To determine foodborne inorganic arsenic exposures worldwide, this research used World Health Organization estimates of food consumption in 13 country clusters, in conjunction with reported measurements of total and inorganic arsenic in different foods. The author estimated slope factors for arsenic-related bladder and lung cancers and used the US Environmental Protection Agency skin cancer slope factor, to calculate the annual risk of the cancer incidence in males and females within each country cluster. The research estimated that each year 9,129 to 119,176 additional cases of bladder cancer, 11,844 to 121,442 cases of lung cancer and 10,729 to 110,015 cases of skin cancer worldwide are attributable to inorganic arsenic in food. These estimates indicate that foodborne arsenic exposure causes a significant global burden of human disease.

4.2 INTRODUCTION

Arsenic is an environmental toxicant naturally found in drinking water and certain foods. The International Agency for Research on Cancer (IARC) classifies arsenic as a Group 1 carcinogen based on evidence that inorganic arsenic causes bladder, lung and non-melanoma skin cancer in humans (IARC, 2012). Arsenic exposure increases risk of mortality from cardiovascular (Chen et al., 2011; Moon et al., 2012) and respiratory diseases (Parvez et al., 2010; von Ehrenstein et al., 2005).

Naturally-occurring levels of arsenic in vegetables, grains, meats and fish present a significant source of arsenic exposure worldwide (Schoof et al., 1999; Kile et al., 2007; Davis et al., 2012). The arsenic comes from uptake by food crops from the soil and irrigation water (Schoof et al., 1999; Samal et al., 2011; Dittmar et al., 2010; Biswas et al., 2012; Muñoz et al., 2001). Arsenic in water can contaminate food during processing and cooking (e.g., in boiling rice, making breads or pasta) (Kile et al., 2007; Signes et al., 2008). According to a recent World Health Organization (WHO) background document on global arsenic exposure (WHO, 2011), arsenic in contaminated water is completely bioavailable and provides the majority of daily arsenic dose (Abernathy et al., 2003). However, as arsenic concentrations in water decrease, the relative contribution of dietary sources becomes more significant to human arsenic exposures (Kile et al., 2007; Davis et al., 2012; EFSA, 2009).

As indicated by its IARC classification, arsenic exposure increases the risk for a number of important cancers. Numerous epidemiological studies indicate an association between arsenic exposure and an increased risk for lung cancer mortality (IARC, 2012; Gibb et al., 2011; Smith et al., 1998; Smith et al, 2009; Ferreccio et al., 2013) and lung cancer may be the leading cause

of arsenic-associated cancer deaths. Meta-analysis of available epidemiological studies performed in Bangladesh, Chile, Argentina, Taiwan and the United States (Begum et al., 2012), estimated about 4.51 additional lung cancer cases per 100,000 people for a maximum level of 10μg/L of arsenic in drinking water found in the studies. An association between arsenic exposure and bladder cancer has been substantiated by multiple ecological, as well as case-control and cohort studies (see IARC, 2012; Gibb et al., 2011; Smith et al., 1998; Christoforidou et al., 2013 for details). An extensive body of literature definitively links the ingestion of arsenic to increased incidence of non-melanoma skin cancer, i.e., basal cell and squamous cell carcinoma (IARC, 2012). Multiple ecological studies based on mortality from skin cancer in Chile, Taiwan, and Bangladesh found consistent gradients of increasing risk with average level of arsenic in drinking water (IARC, 2012; Hughes et al., 2011). Cohort studies from IARC (2012) reported risks of skin cancer to be significantly related to average concentration of arsenic in drinking water and index for cumulative exposure to arsenic (Hughes et al., 2011; Wu et al., 1989; Hsueh et al. 1995).

The objective of the study being presented was to use quantitative risk assessment to estimate the global burden of foodborne arsenic-induced bladder cancer, lung cancer and skin cancers. Global burden of disease (GBD) is a widely accepted parameter that provides a frame of reference for comprehensive analysis of health gaps. It relies on the use of all available mortality and health data by appropriate methods to confirm the comparability and consistency of estimates of demographic and epidemiological importance worldwide. This risk estimate was made as part of the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) efforts to estimate the GBD from foodborne chemical exposures, including dietary inorganic arsenic exposure. A partial risk assessment was made previously by the Joint FAO/WHO Expert Committee on Food

Additives (JECFA) who reviewed the PTWI of inorganic arsenic with an emphasis on the speciation and occurrence of inorganic arsenic in food (JECFA, 2011). In addition, the human health risks from foodborne arsenic in European countries were assessed by the EFSA Panel on Contaminants in the Food Chain (EFSA, 2009). However, the global burden of cancers caused by foodborne arsenic exposure has not been investigated, nor the extent of inorganic arsenic content in different diets worldwide.

This study focused on adverse effects associated with inorganic arsenic exposure, since foodborne organic arsenical exposures pose little human health risk (Kile et al. 2007; Davis et al., 2012; Hughes et al., 2011; JECFA, 2011; EFSA 2009). The author estimated the number of additional cases of cancers per year due to inorganic arsenic through food in different diets worldwide, based on data adapted from WHO Global Environment Monitoring System (GEMS)/Food Consumption Cluster Diets database (WHO GEMS, 2006). GEMS/Food Consumption Cluster Diets database divides the countries of the world into 13 groups based on diets.

4.3 RESULTS

The essential steps of risk assessment are hazard identification, dose-response relationship, exposure assessment and risk characterization. The present work relied on hazard identification by IARC (IARC, 2012) that clearly identifies arsenic as a human carcinogen with increased risk for bladder, lung and non-melanoma skin cancers. The dose response estimates were converted for water exposure to human dose to establish the dose-response relationship. Table 7 includes

the imputed slope factors for each of the cancers. For bladder and lung cancers gender-specific slope factors are reported based on the data adapted from Morales et al. (2000). For skin cancer, the slope factors are the same for both genders (USEPA IRIS, 1998). The total increased risk in the population of each cancer for every incremental unit of foodborne arsenic was estimated on the basis of the slope factors.

Table 7 - Slope factors, or cancer potency factors, for incidence of each arsenic-related cancer

Cancer type	**Slope factor (increased population risk per µg inorganic arsenic/day)**	
	Males	***Females***
Bladder*	0.0000127	0.0000198
Lung*	0.0000137	0.0000194
Skin^	0.000015	0.000015

***** slope factor derived by using data adapted from Morales et al. (2000)

^ slope factor was adapted from the United States EPA IRIS Database (2001)

For exposure estimation, Table 8 provides the mean adjusted total arsenic content of foods used in the EFSA (2009) dietary exposure estimates along with the conversion factors from total arsenic to inorganic arsenic in each of the different foodstuffs provided in JECFA (2011). In contrast to water exposures, not all of the arsenic in food is bioavailable and Table 9 presents the estimated levels of bioavailable inorganic arsenic for the 13 GEMS food consumption clusters as well as the population size for each cluster. For each of these clusters, the GEMS food consumption database provides an estimate of the amount of cereals, vegetables, fruits, beverages, meat, nuts and oilseeds consumed. Rice and rice products appear to be a major source of exposure to inorganic arsenic, especially in GEMS cluster G, comprised of Asian countries.

Risk characterization of the total estimated cases of bladder, lung and skin cancers attributable to foodborne arsenic annually worldwide was calculated from the slope factors in Table 7 and the exposure data in Tables 8 and 9. These estimates are listed in Table 10 and can be further resolved by GEMS cluster and gender to yield the number of expected additional cases of bladder, lung and skin cancer from foodborne inorganic arsenic exposures per year. Table 11 presents these cases with the assumption of 70 years life span per individual. Overall, the data indicate that arsenic in food causes a small but significant burden of the three major cancers that is distributed throughout the world.

Table 8 - Mean adjusted total arsenic content of foods and the reported conversion factors from total arsenic to inorganic arsenic used in the dietary exposure estimates.

Food group	Total arsenic lower bound mean level (mg/kg)	Total arsenic upper bound mean level (mg/kg)	Mean % inorganic Arsenic
01. All cereal & cereal products	0.0671	0.0848	30–100^
01.A Cereal-based dishes	0.0157	0.0283	
01.B Cereal & cereal products	0.0825	0.1017	
02. Sugar products and chocolate	0.0135	0.0320	30-100^
03. Fats (vegetable and animal)	0.0063	0.0245	30-100^
04. All vegetables, nuts, pulses	0.0121	0.0212	30-100^
04.A Vegetable soups	0.0050	0.0110	
04.B Vegetables, nuts, pulses	0.0122	0.0213	
05. Starchy roots and tubers	0.0031	0.0142	30-100^
06. Fruits	0.0051	0.0155	30-100^
07. Juices, soft drinks and bottled water	0.0030	0.0068	30-100^
07.A Fruit and vegetable juices	0.0048	0.0129	
07.B Soft drinks	0.0044	0.0132	
07.C Bottled water	0.0023	0.0041	
08. Coffee, tea, cocoa	0.0034	0.0051	30-100^
09. Alcoholic beverages	0.0055	0.0151	30-100^ [this category not detailed in GEMS diets
09.A Beer and substitutes	0.0054	0.0161	
09.B Wine and substitutes	0.0061	0.0110	

09.C Other alcoholic beverages	0.0085	0.0155	database and hence was not used for calculations]
10. All meat and meat products, offal	0.0044	0.0138	100*
10.A Meat and meat products	0.0042	0.0137	
10.B Edible offal and offal products	0.0044	0.0139	
10.C Meat-based preparations	0.0121	0.0185	
11. All fish and seafood	1.6136	1.6159	*Standard ratio* 0.015 – 0.10 mg/kg^
11.A Seafood and seafood products	5.5537	5.5545	
11.B Fish and fish products	1.4426	1.4549	
11.C Fish-based preparations	1.1524	1.1573	
12. Eggs	0.0042	0.0117	41*
13. Milk and milk-based products	0.0044	0.0139	26*
13.A Milk and dairy-based drinks	0.0026	0.0104	
13.B Dairy-based products	0.0068	0.0184	
13.C Cheese	0.0065	0.0188	
14. Miscellaneous/special dietary products	0.3993	0.4187	30-100^ Category not detailed in GEMS
14.A Miscellaneous products	0.2449	0.2658	
14.B Foods for special dietary uses	0.4383	0.4573	

^ *Data adapted from EFSA (2009) and FAO/WHO JECFA Monographs 8, 2011*

* *Reference: Yost et al. (1998)*

Table 9 - Range of foodborne total and inorganic arsenic exposure at 50 -100% bioavailability for 13 WHO - GEMS clusters of countries[¥]

GEMS Cluster	Lower boundary of total As* (µg/kg bw/ day)[a]	Upper boundary of total As* (µg/kg bw/day)	Lowest boundary of inorganic arsenic[b] (50% bioavailable) (µg/day)^	Upper boundary of inorganic arsenic[c] (100% bioavailable) (µg/ day)^	Range of inorganic arsenic exposure via rice and rice products (µg/ day)	Population mid-2012 (millions)[#]
A	0.91	1.26	4.8	53.4	0.92 to 6.95	302.5
B	2.87	3.47	10.37	108.35	0.32 to 2.41	224.9
C	1.38	1.79	9.09	85.46	0.95 to 7.22	263.7
D	1.32	1.72	6.71	66.95	0.33 to 2.53	408
E	1.41	1.83	5.75	63.45	0.13 to 0.97	339.2
F	1.84	2.19	5.25	57.27	0.13 to 0.97	26.7
G	2.08	2.42	7.82	75.14	3.79 to 28.78	3544.5
H	1.15	1.55	6.44	66.54	0.65 to 4.9	213.5
I	0.87	1.18	5.02	52.2	0.38 to 2.9	256.8
J	0.97	1.28	5.01	51.88	0.75 to 5.67	357
K	1.04	1.48	6.6	66.13	2.39 to 18.19	335.7
L	2.69	3.05	7.88	79.1	3.84 to 29.1	307.4
M	1.35	1.83	6.44	70.56	0.35 to 2.64	436.8

Data adapted from GEMS/Food Consumption Cluster Diets database (FAOSTAT 2006)

[¥] Listing of countries within each cluster is available at http://www.who.int/foodsafety/chem/gems/en/index1.html.

[a] Assuming 60 kg body weight per individual

[b] Lower bound for inorganic arsenic content assumes Non detect equals zero

[c] Upper bound for inorganic arsenic content assumes non-detect equals the limit of detection

* Calculations based on Table 13, FAO/WHO JECFA Monographs 8, 2011 for range of total arsenic content in food items.

^ Calculations based on Table 15, FAO/WHO JECFA Monographs 8, 2011 for range of mean % inorganic arsenic content in food items.

Data source: "Population Data sheet 2012" by the Population Reference Bureau (www.prb.org). PRB has derived the data from International Programs Center of the US Census Bureau; the United Nations (UN) Population Division; the Institut national d'etudes démographiques (INED), Paris; and the World Bank.

Table 10 - Global burden of cancers caused by foodborne arsenic

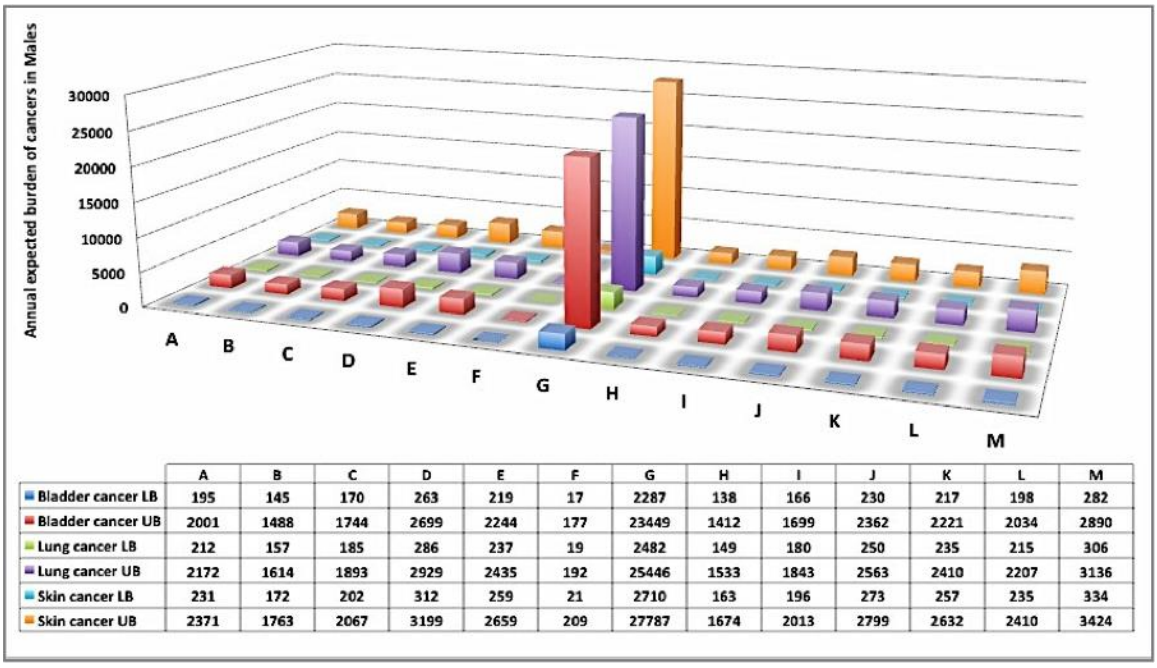
Cancer	Male	Female	Total burden (global) by foodborne arsenic
Bladder	4,527 to 46,420	7,096 to 72,756	9,129 to 119,176
Lung	4,913 to 50,373	6,931 to 71,069	11,844 to 121,442
Skin (Non melanoma)	5,365 to 55,007	5,365 to 55,007	10,730 to 110,014

Table 11 - Annual expected burden of cancers caused by foodborne arsenic, by GEMS cluster and gender, lower bounds (LB) and upper bounds (UB)[a]

GEMS cluster	Bladder cancer				Lung cancer				Skin cancer			
	Male		*Female*		*Male*		*Female*		*Male*		*Female*	
	LB	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
A	195	2001	306	3137	212	2172	299	3064	231	2371	231	2371
B	145	1488	227	2332	157	1614	222	2278	172	1763	172	1763
C	170	1744	267	2734	185	1893	260	2671	202	2067	202	2067
D	263	2699	413	4230	286	2929	403	4132	312	3199	312	3199
E	219	2244	343	3517	237	2435	335	3436	259	2659	259	2659
F	17	177	27	277	19	192	26	270	21	209	21	209
G	2287	23449	3584	36753	2482	25446	3502	35901	2710	27787	2710	27787
H	138	1412	216	2214	149	1533	211	2162	163	1674	163	1674
I	166	1699	260	2663	180	1843	254	2601	196	2013	196	2013
J	230	2362	361	3702	250	2563	353	3616	273	2799	273	2799
K	217	2221	339	3481	235	2410	332	3400	257	2632	257	2632

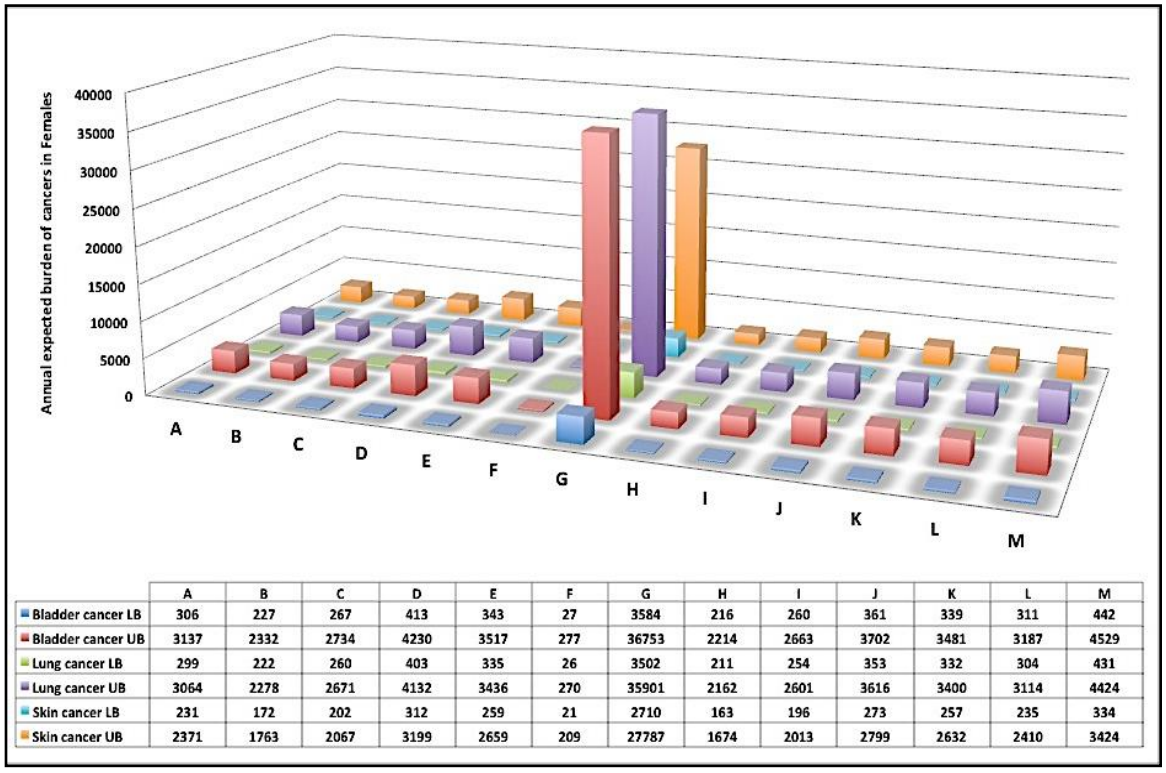
L	198	2034	311	3187	215	2207	304	3114	235	2410	235	2410
M	282	2890	442	4529	306	3136	431	4424	334	3424	334	3424
Total	4527	46420	7097	72756	4913	50373	6932	71069	5365	55007	5365	55007

[a]Assuming 70 years life span per individual



	A	B	C	D	E	F	G	H	I	J	K	L	M
Bladder cancer LB	195	145	170	263	219	17	2287	138	166	230	217	198	282
Bladder cancer UB	2001	1488	1744	2699	2244	177	23449	1412	1699	2362	2221	2034	2890
Lung cancer LB	212	157	185	286	237	19	2482	149	180	250	235	215	306
Lung cancer UB	2172	1614	1893	2929	2435	192	25446	1533	1843	2563	2410	2207	3136
Skin cancer LB	231	172	202	312	259	21	2710	163	196	273	257	235	334
Skin cancer UB	2371	1763	2067	3199	2659	209	27787	1674	2013	2799	2632	2410	3424

Figure 5 - Annual expected burden of cancers caused by foodborne inorganic arsenic, by GEMS cluster and sex, lower bounds (LB) and upper bounds (UB) in males



	A	B	C	D	E	F	G	H	I	J	K	L	M
Bladder cancer LB	306	227	267	413	343	27	3584	216	260	361	339	311	442
Bladder cancer UB	3137	2332	2734	4230	3517	277	36753	2214	2663	3702	3481	3187	4529
Lung cancer LB	299	222	260	403	335	26	3502	211	254	353	332	304	431
Lung cancer UB	3064	2278	2671	4132	3436	270	35901	2162	2601	3616	3400	3114	4424
Skin cancer LB	231	172	202	312	259	21	2710	163	196	273	257	235	334
Skin cancer UB	2371	1763	2067	3199	2659	209	27787	1674	2013	2799	2632	2410	3424

Figure 6 - Annual expected burden of cancers caused by foodborne inorganic arsenic, by GEMS cluster and sex, lower bounds (LB) and upper bounds (UB) in females

Correction of cancer burden estimates for age

The estimates for global burden of cancers attributable to foodborne arsenic are expected to reflect the age distribution for the incidence of these cancer types in general. Arsenic is not an independent carcinogen, rather it is a co-carcinogen requiring other genotoxic events to initiate cancer. It is known to enhance cancer progression. As such, being exposed to arsenic might

predispose an individual to develop cancer upon a subsequent or simultaneous exposure to a potential carcinogen. Human exposure to environmental toxins gradually accumulates with the progression of age and this is expected to apply to foodborne arsenic as well.

Age has a powerful influence on the risk of cancer, so age-standardization is necessary when comparing several populations that differ with respect to age. An age-standardized rate (ASR) is a summary measure of the rate that a population would have if it had a standard age structure (Globocan, 2008). Age-standardized world incidence/mortality estimates for cancers were obtained from the Globocan database which is a part of the section of cancer Information (CIN) of IARC. In Globocan, the ASR is calculated using 10 year age-groups. For bladder and lung cancers, data on age-standardized rates (per 100,000) for both genders depicts that the incidence rate increases with an increase in the age groups of the population. The ASR values increase for both lung and bladder cancers in the age groups above 55 years. Morales et al. (2000) note in relation to standard mortality rates of cancers that: *"There is no observed tendency in SMRs with respect to age, which suggests no age dependency on the risk ratio."*

The calculations of age-specific onset of disease caused by arsenic exposure would ideally require epidemiological data with age-specific incidence by dose. Gibb et al. (2011) suggested that additional follow-up of the northeastern Taiwanese cohort and the HEALS cohort in Bangladesh could provide this crucial piece of information for future analysis.

For the current analysis of age-specific incidence of bladder and lung cancers, data was adapted from Morales et al. (2000), Table 2 (reproduced in Appendix B) that provides a comparison of population data from all of Taiwan and southwestern Taiwan, with sex and age-wise distribution of the person years at risk (PYR) and mortality due to bladder, lung and liver cancers. This data

can be used to find age-distribution for arsenic-induced bladder and lung cancers because all Taiwan and southwestern Taiwan have populations that are almost identical in all respects except for differences in exposure to arsenic in drinking water. The data from Morales et al. (2000) was used to assess the differences in age-distribution of occurrence of bladder and lung cancers in the two populations from all Taiwan and southwestern Taiwan. As the values of PYR in each age group are different for all Taiwan and southwest Taiwan, these values were normalized such that the total is equal to 100. The differences in age distribution of total bladder and lung cancer cases were then calculated to estimate the age-specific burden by arsenic. Table 12 depicts the estimates of the annual number of arsenic-induced bladder and lung cancer cases per age group.

For non-melanoma skin cancer, data was adapted from Tseng (1977) Table 1 (reproduced in Appendix A) that details the age-specific and sex-specific prevalence rate for skin cancer in southwestern Taiwan. The percentage-wise distribution of NMSC cases due to arsenic was then computed by normalizing the values from the column "number of cases per 1000" for males/ females such that the total is 100 (Table 13).

Global estimates of age-specific incidence of expected additional cases of bladder cancer, lung cancer and skin cancer from foodborne inorganic arsenic exposures per year are presented in Table 14**.** Figures 2 and 3 present the age-specific incidence for bladder, lung and skin cancer cases due to arsenic in food in males and females respectively.

Table 12 - Relationship of age and sex to additional annual bladder and lung cancer cases attributable to arsenic

Sex, age (years) Males	Bladder cancer			Lung cancer		
	All Taiwan	SW Taiwan	Additional cases in SW Taiwan compared to All Taiwan (Normalized)	All Taiwan	SW Taiwan	Additional cases in SW Taiwan compared to All Taiwan (Normalized)
20-25	0.0	0.0	0.2	0.0	0.1	0.8
25-30	0.0	0.1	0.5	0.1	0.2	2.1
30-35	0.0	0.1	0.1	0.2	0.3	2.9
35-40	0.1	0.2	0.5	0.3	0.4	1.4
40-45	0.4	0.5	1.9	0.7	0.8	4.5
45-50	0.7	0.9	4.0	1.4	1.7	7.1
50-55	1.2	1.6	4.9	2.9	3.3	10.2
55-60	1.9	2.3	5.4	5.5	6.0	15.1
60-65	4.3	5.2	12.4	9.3	9.9	17.8
65-70	7.7	9.1	18.2	13.9	14.3	10.0
70-75	12.8	15.3	31.4	17.2	17.9	19.50
75-80	19.3	20.8	20.5	17.5	17.8	8.6
80-85	23.1	19.0	0	17.1	15.6	0
85+	28.5	24.9	0	13.8	11.9	0

Females						
20-25	0	0	0	0.1	0.8	6.9
25-30	0.0	0.1	0.6	0.1	0.3	1.6
30-35	0.0	0	0	0.3	0.4	0.9
35-40	0.1	0.1	0.4	0.7	1.0	3.1
40-45	0.4	0.6	1.4	1.4	1.9	5.0
45-50	0.9	1.0	1.1	2.2	3.1	7.8
50-55	1.9	3.1	9.7	3.6	5.1	13.0
55-60	4.0	5.5	13.2	6.3	7.8	14.3
60-65	6.3	9.9	32.6	9.5	12.3	25.5
65-70	9.3	11.3	17.6	13.2	14.2	9.3
70-75	14.1	16.7	23.3	15.4	16.8	12.6
75-80	20.1	17.7	0	17.2	16.4	0
80-85	21.3	17.9	0	16.5	14.2	0
85+	21.4	16.0	0	13.5	6.6	0

Table 13 - Expected percent of non-melanoma skin cancer cases attributable to arsenic, by age group

Age (years)	Males		Females	
	Per 1000	Percent of cases due to arsenic (by age-group)	Per 1000	Percent of cases due to arsenic (by age-group)
0-19	-	0	-	0
20-29	1.0	0.2	1.1	0.7
30-39	9.7	2.1	1.5	0.9
40-49	25.9	5.7	8	5.3
50-59	80.8	17.8	28.9	19.2
60-69	124.8	27.6	57.0	37.9
70+	209.6	46.4	53.8	35.8

Table 14 - Annual expected age-specific incidence of bladder and lung cancers caused by foodborne arsenic compared on the basis of gender and range of exposure

Age (years)	Bladder cancer				Lung cancer			
	Males		*Females*		*Males*		*Females*	
	LB	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
20-25	9	91	0	0	37	383	479	4909
25-30	21	214	27	434	105	1080	107	1100
30-35	3	33	0	0	141	1445	63	643
35-40	22	222	19	302	70	713	213	2189
40-45	90	922	65	1022	222	2274	348	3568
45-50	182	1865	52	828	350	3585	541	5552
50-55	223	2286	444	7017	501	5132	903	9261
55-60	243	2498	609	9632	742	7608	992	10172
60-65	562	5763	1502	23739	875	8974	1769	18134
65-70	824	8455	811	12823	489	5015	641	6571
70-75	1421	14571	1073	16959	958	9823	875	8970
75-80	927	9500	0	0	423	4341	0	0
80-85	0	0	0	0	0	0	0	0
85+	0	0	0	0	0	0	0	
Total	4527	46420	4602	72756	4913	50373	6931	71069

Table 15 - Annual expected age-specific incidence of non-melanoma skin cancers caused by foodborne arsenic (gender and range of exposure)

Age (years)	Non-melanoma skin cancer			
	Males		*Females*	
	LB	**UB**	**LB**	**UB**
0-19	0	0	0	0
20-29	12	122	12	122
30-39	115	1181	115	1181
40-49	308	3153	308	3153
50-59	959	9837	959	9837
60-69	1482	15195	1482	15195
70+	2489	25519	2489	25519
Total	5365	55007	5365	55007

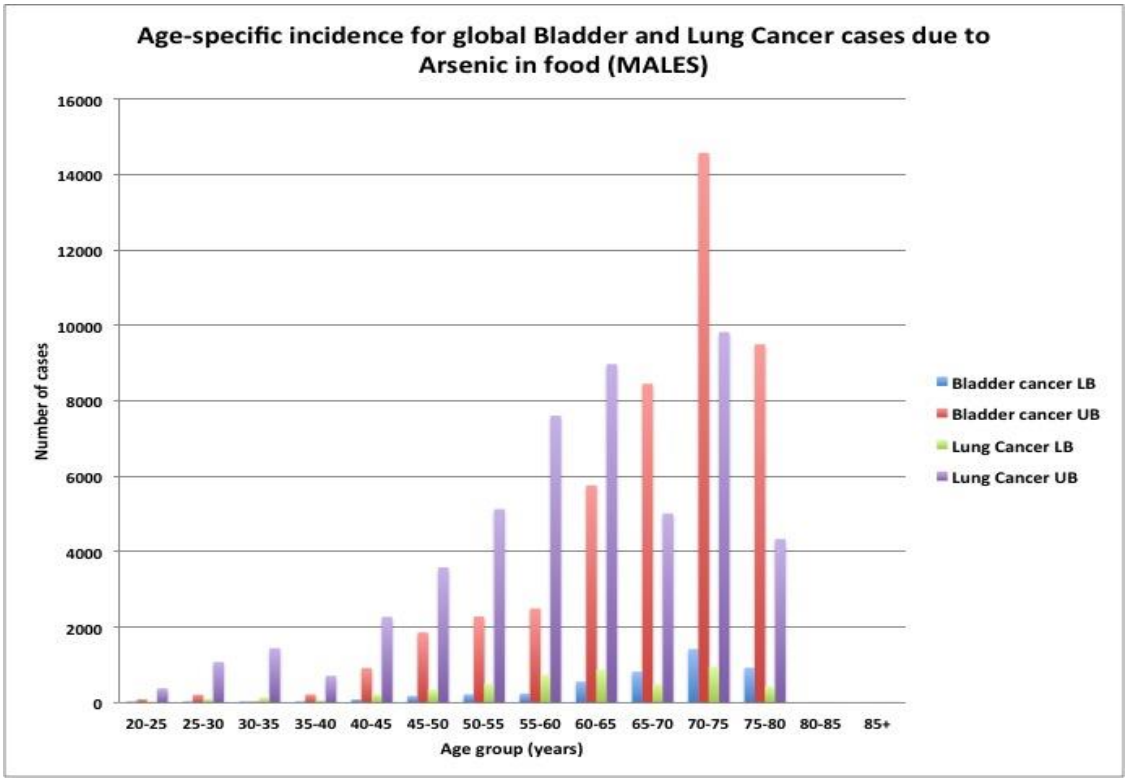


Figure 7 - Males: Age-specific incidence for bladder and lung cancer cases due to arsenic in food

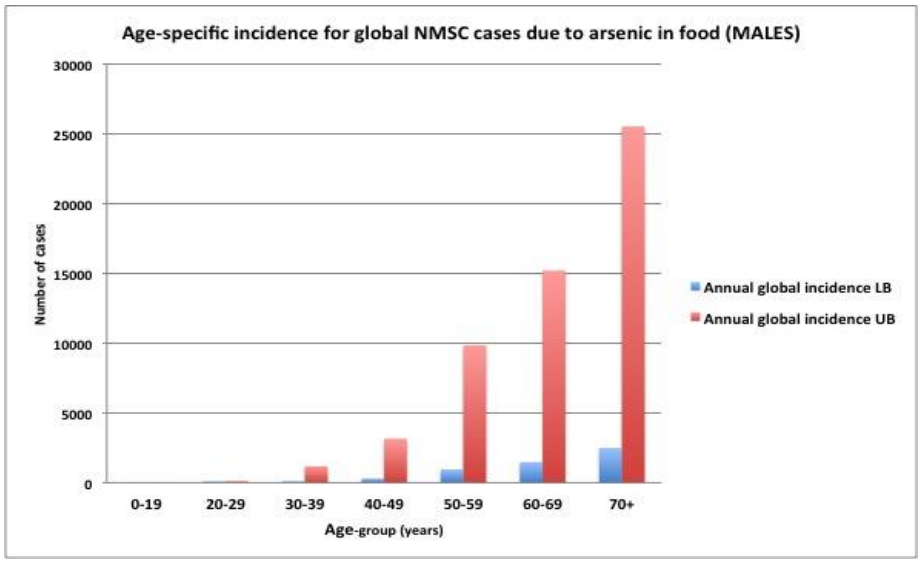


Figure 8 - Males: Age-specific incidence for non-melanoma skin cancer cases due to arsenic in food

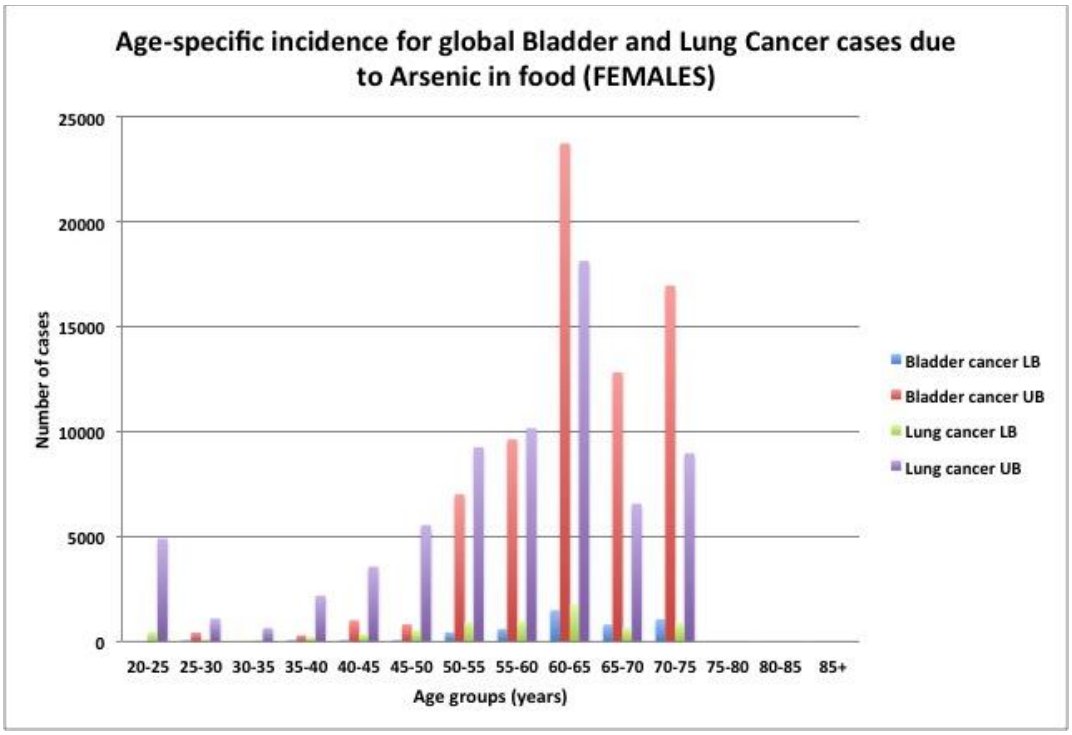


Figure 9 - Females: Age-specific incidence for bladder and lung cancer cases due to arsenic in food

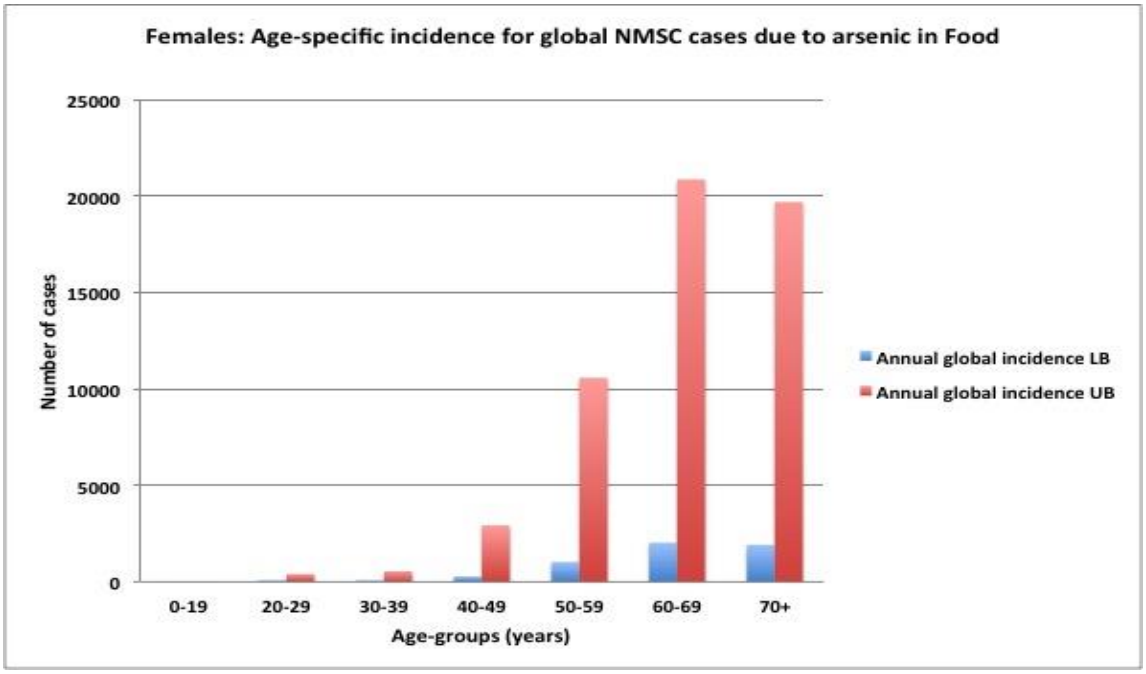


Figure 10 - Females: Age-specific incidence for non-melanoma skin cancer cases due to arsenic in food

4.4 DISCUSSION

Using quantitative risk assessment, this research estimated the increased incidence of cancers that can be attributed to arsenic in food. The most challenging aspect was estimating the highly variable levels of inorganic arsenic in the varied foods consumed by the different populations contained in the GEMS clusters. There is uncertainty in whether arsenic in food is equivalent to

arsenic in water for disease promotion given the many other food constituents, such as folate (Hall et al., 2012) and selenium (Chen et al., 2007) that may modulate arsenic pathogenesis. The assumption of linear dose-response relationships of arsenic-related cancers is controversial, particularly regarding the mode of carcinogenicity of skin cancer, despite the EPA IRIS derivation of a single slope factor for arsenic-related skin cancer (USEPA IRIS, 1998). There are no studies that present the effects of low dose arsenic exposures on skin cancer, which reduces certainty regarding the shape of the lower end of the dose-response curve. Thus it is conservative to default to the linear model for determining the skin cancer potency factor. Accounting for these uncertainties, this dissertation estimates that levels of inorganic arsenic found in food cause a low but significant increase in the burden of lung, bladder, and non-melanoma skin cancers worldwide.

Much of the available data on disease risk come from studies of arsenic in drinking water and often the populations studied have been exposed to higher levels of arsenic (>100 μg/L drinking water). As levels of arsenic in water decrease, the contribution of arsenic from food to total arsenic exposure becomes greater and more significant (Kile et al., 2007; Kurzius-Spencer et al., 2014). While human biomarkers for arsenic exposure (such as arsenic and metabolite levels in urine, blood, hair, or nails) are available (Kurzius-Spencer et al., 2014), it is not possible to determine the proportion of the measurements attributable to arsenic in drinking water or food. For the purposes of estimating human health consequences associated with arsenic consumption, knowing the overall population arsenic exposure matters more than knowing the relative contribution from different routes of exposure. For the purpose of recommending interventions, it can be helpful to understand the separate contributions.

There are several additional unavoidable constraints with estimating health risks from arsenic in food. The bioavailability of arsenic in different foods varies with the food group or method of processing and the complexity of influence of other food constituents on arsenic toxicity and adverse health effects. The author focused the exposure estimates and risk characterization on both the range of inorganic arsenic content and the range of predicted bioavailability of inorganic arsenic in different foods. This approach is limited to using the GEMS cluster data for food consumption, since it contains an inherently broad range of dietary variations between the countries within each cluster (Liu et al., 2010). For example, the daily consumption of rice in Bangladesh (GEMS cluster G country) was reported as 445g/day (Meharg et al., 2009), even when the average rice consumed daily for GEMS cluster G is 380g. Using the cluster values may underestimate the arsenic exposure via rice in Bangladesh; on the other hand, while the actual daily consumption overall for cluster M is 35g/day, for the USA (GEMS cluster M country) it is 18 g (Meharg et al., 2009). One of the major assumptions in the current analysis is that the speciation and arsenic content of rice cultivated in different regions of the world would be the same. Nevertheless, there are conflicting reports indicating a large variation in the levels of inorganic arsenic in rice from developing and developed countries (Meharg et al., 2009; Carey et al., 2011). In order to overcome these limitations and obtain a realistic estimate for inorganic arsenic levels, the author used data from studies that provide actual measured levels (EFSA, 2009) in different categories of food items (Schoof et al., 1999; Muñoz et al., 2001; Diaz et al., 2012).

The GEMS cluster data do not provide specific details of the consumption of certain miscellaneous food items with reported high levels of inorganic arsenic (e.g., seaweed hijiki and edible algae (EFSA, 2009) Table 2, miscellaneous items). In certain Asian countries, such as

Japan, the consumption of seaweed is a relatively important part of diet and can add substantially to the daily exposure levels of inorganic arsenic (JECFA, 2011; Uneyama et al., 2007).

Despite the complexity of assessing foodborne arsenic exposures, the estimates for global burden of cancers caused by the estimated range of exposures appear feasible. The research presented here found that human exposures to inorganic arsenic through food is substantial (see Table 8) and can be roughly comparable with lower levels of arsenic in drinking water. It was reasonable to convert the data from that of Morales et al. (2000) to dietary consumption and calculate the slope factors for lung and bladder cancers to estimate the risk of foodborne inorganic arsenic. This data set reduces the concern about issues of low-dose extrapolations of arsenic's carcinogenic effects, although the estimates would be improved by including additional epidemiological studies that focus on low dose consumption.

The review by Gibb et al. (2011) emphasized the need for such studies on bladder and lung cancer that address adequacy of the sample size, as well as the synergistic relationship of arsenic and smoking, duration of arsenic exposure, age when exposure began and ended and the histologic subtype of cancer. They observed that many recent studies that examine the risk ratio of bladder cancer from low arsenic concentration (<100μg/L) drew cases and controls from arsenic-endemic areas that may reduce the difference in arsenic exposure, requiring a larger sample size to determine whether an excess risk exists for a given exposure. The potential for smoking to confound the risk estimates attributable to dietary arsenic exposure would likely be true for lung cancer estimates as well. The exposure misclassification further reduced the difference between groups and epidemiological studies focused on low-arsenic levels have a greater need to control for confounders (Gibb et al., 2011).

The estimated global burden for arsenic-induced bladder and lung cancers is the highest for both males and females in cluster G for several reasons. Firstly, cluster G comprises of countries in Asia where the arsenic content in the bedrock ranks among the highest in the world. This translates into high overall rate of exposure to arsenic through more than one route of exposure and on a consistent basis for an extended period- thus pre-disposing this population to develop arsenic-induced cancers. Secondly, rice is the main food consumed in most of the countries in Cluster G. Table 3 shows that rice contributes up to 68.1% of inorganic arsenic exposure in cluster G countries. Related to the first reason or type of cultivar, rice grown in cluster G contains higher levels of arsenic than rice grown elsewhere (Meharg et al., 2009; Carey et al., 2012). Finally, the population size is a chief component in the model for the estimation of the disease burden. Cluster G comprises nearly 50% of the world population, including China and India. Therefore, although the percentage of arsenic via rice is high in cluster L countries as well (up to 65.8%), it does not reflect in a high global burden of disease for this cluster owing to its small population size.

In conclusion, the results of this quantitative risk assessment indicate that consumption of arsenic in food increases the risk of bladder, lung and skin cancer. There are limitations with the estimates that are derived from the ranges of arsenic content in food and the interactions of arsenic with other foodborne constituents. Nonetheless, the risk estimates are valuable for informing policies to reduce the global burden of disease from arsenic exposures in food.

5.0 GLOBAL BURDEN OF DISEASE FOR CORONARY HEART DISEASE CAUSED BY ARSENIC IN FOOD

5.1 ABSTRACT

Cardiovascular disease, especially coronary heart disease (CHD), is one of the most prominent non-cancer disease risks clearly linked to environmental arsenic exposures. The cardiovascular effects of oral arsenic exposure are a global public health concern causing disease in millions of people worldwide. As the levels of arsenic in water become universally regulated, the exposure through food sources becomes critical in promoting disease development. This research estimates a reference dose for arsenic-induced CHD and provides an estimated burden of coronary heart disease due to inorganic arsenic exposure through food. A range of inorganic arsenic content in food for different parts of the world was estimated earlier using World Health Organization estimates of food consumption in thirteen country clusters and the arsenic content measurements of different food items reported in literature. A benchmark dose was derived from a relevant study in mice of arsenic-induced atherosclerosis, the main clinical driver of CHD. Reference doses for dietary arsenic intake and CHD and atherosclerosis were developed. The benchmark dose for CHD was also used to estimate the additional burden of disease for CHD attributable to arsenic in food. These estimates indicate that foodborne arsenic exposure poses a significant risk and may contribute to global burden of coronary heart disease in humans at the upper boundaries of arsenic exposure from food.

5.2 INTRODUCTION

Oral exposure to inorganic arsenic through water and food is a prominent global health problem (EFSA, 2009). The risk for cardiovascular diseases has been observed to increase in areas with high arsenic levels in drinking water (Moon et al., 2012). Given the high burden of cardiovascular disease worldwide, cardiovascular disease is likely to be the most important non-cancer disease risk posed by environmental arsenic exposures (NRC, 2013). Limited studies exist for cardiovascular effects of low to moderate arsenic levels (<150 μg/L in drinking water). The absence of strong prospective studies, limitations in assessment of exposure and outcome and incomplete details on cardiovascular risk factors have been major concerns with a number of studies (Navas-Acien et al., 2005; Moon et al., 2013). The Strong Heart Study designed to obtain clinical data on cardiovascular disease in Native Americans was successful in determining the lower end of the dose response relationships for arsenic-promoted CHD incidence and mortality (Moon et al., 2013).

High arsenic content in drinking water is undeniably the most important route of exposure in many parts of the world. Until recently little research was focused on food components as a potential source of arsenic exposure. There has been an increasing interest in analyzing the harmful health effects of foodborne arsenic with increasing evidence about the tendency of dietary components (e.g. rice) to accumulate arsenic from soil and irrigation water. Since rice is a dietary staple in many parts of the world, its arsenic content only adds to the public health significance of such exercises. Global food trade also necessitates such research because anybody can be at the risk of exposure to arsenic from food cultivated with high arsenic content in the opposite corner of the world.

CHD refers to the disease of the coronary arteries and the resulting complications of myocardial infarction, angina, and ultimately cardiac death. Most clinical manifestations of CHD are caused by atherosclerosis. The objective of the research presented here was to use BMD analysis to estimate the global burden of foodborne arsenic-induced coronary heart disease. Global burden of disease due to specific toxicant exposure provides key information to policy formulators for making knowledge-based decisions on the most effective means to reduce or prevent disease. This research provides reference dose values for arsenic in food to determine the relative risk of CHD from food, as well as BMD values that lead to estimation of additional cases of CHD in different parts of the world consuming different amounts of arsenic in foods. This research work contributes towards providing a comparison of BMD values for CHD based on epidemiological and animal data for atherosclerosis.

5.3 METHODS

As described in Chapter 2, BMD modeling was used to define a point of departure that is largely independent of study design. BMD was estimated by the fitting of various mathematical models to the observed data using USEPA BMD software. The current version of the USEPA BMD software (version 2.4) is more suitable for analyzing animal studies. Animal data avoid issues with confounding variables. Conventionally it is less common for the derivation of BMD to be based on epidemiological data primarily because for many compounds, epidemiological studies of adequate quality are not available. For arsenic, recent epidemiologic literature was reviewed to identify strong epidemiological studies that provide dose-response information that was adjusted for the main potential confounding factors associated with CHD.

5.3.1 Studies selected for BMD calculation

This section has been described in part in the Chapter 2 on Methods with the exception of the detailed methodology for primary data on mouse studies, which is presented here.

In Vivo Mouse Exposure and Atherosclerotic Lesion Characterization

The mouse exposure studies and assessment of atherosclerotic plaques were conducted by Dr. Maryse Lemaire in the laboratory of Dr. Koren Mann (Lady Davis Institute for Medical Research, McGill University, Montreal, Canada). The exposures were performed in agreement with the respective institutional guidelines for animal safety and welfare under the supervision of the McGill Animal Use Committee. Male $ApoE^{-/-}$ mice (Taconic Farms, Hudson, NY) (5 weeks old, $n \geq 5$) were either maintained on tap water or on tap water-containing sodium meta-arsenite ($NaAsO_2$) (Sigma, MO). The $ApoE^{-/-}$ mouse model was used because these mice develop atherosclerotic plaques, in contrast to wild-type mice, providing a validated tool for atherosclerosis research. Atherosclerotic lesion formation was examined en face in the aortic arch and in cross-sections of the aortic sinus. Mice that received $NaAsO_2$ were exposed to arsenic ranging from 10 ppb to 200 ppb (0.35 mg/l $NaAsO_2$) for 13 weeks. This exposure is representative of a human drinking moderate to highly arsenic contaminated water for 5-8 years. Solutions containing $NaAsO_2$ were refreshed every 2–3 days to minimize oxidation to As(V).

The entire aorta, from the heart to the iliac arteries, was removed and rinsed with PBS and fixed in 4% paraformaldehyde. Periadventitial tissue was removed and the aorta was cut longitudinally. The aortic surface was stained en face with oil red O (Electronic Microscopy Sciences, PA). Lipid staining is not necessary for quantification of atherosclerotic lesions, but it

can be useful in assessing the disease when lesions are small (Daugherty et al., 2003). Percentage of lesion area of the aortic arch, as defined as the region from ascending arch to the first intercostal arteries, was evaluated with the Infinity Analyze software 5.0 (Lumenera, Canada).

The heart was removed, rinsed, fixed in 4% paraformaldehyde and incubated overnight in a 30% sucrose solution (Braun et al., 2003). The tissues were frozen then in Tissue Tek OCT (Sakura, CA) reagent, and serial cryosections of 6μm thickness were cut from the origin of the aortic root throughout the aortic sinus. 5 to 7 sections per animal were stained with oil red O and the mean lesion area was calculated using ImageJ software (National Institute of Health). Percentage of lesion area was evaluated relative to the total aortic sinus area. The lipid content and the collagen content of the plaque were evaluated with ImageJ, using their specific stains (oil red O and picrosirius red (Polysciences, PA), respectively).

Goodness of Fit test statistics

No model was determined to be the most biologically plausible for the endpoints that were considered. All models were found to describe the data well, based on the comparison of *p*-value (> 0.1); however, the Weibull and Gamma models failed to generate a plot. The best-fitting model for dichotomous data was selected consistent with the USEPA *Benchmark Dose Technical Guidance Document* (USEPA BMDS, 2012) from the models exhibiting adequate fit, as follows. If the BMDL estimates from the models exhibiting adequate fit were within a range of two- to threefold, then the model with the lowest AIC was selected. If the range of BMDLs is larger than two- to threefold (indicating that some model dependence is assumed), then the model with the lowest BMDL is selected.

Choice of benchmark response (BMR)

BMD approach begins by identifying a criterion for adverse effect. In most analyses, the criterion for adverse effect has been the manifestation of disease in a non-exposed population. BMD is defined as the level of exposure that will increase the risk of disease by a pre-specified amount. This increase is referred to as the benchmark response (BMR) (Jacobson et al., 2002). For BMD analysis of the epidemiological data, BMR of 1% was selected as it would generate a BMD for an increase of 1% in the disease rate. This would provide a conservative estimate of any increase in disease in the general population over the non-exposed population. For the mice data, a change in the mean equal to one control standard deviation from the control mean was used as BMR, since there is no other over-riding reason for defining an alternative BMR. BMR of one standard deviation (SD) gives an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile or above the 98th percentile of controls for normally distributed data. Therefore $BMD_{1.0SD}$ is the profile-likelihood-based dose for which the response equals a predicted mean one standard deviation below the predicted control mean.

Computation of BMD

Epidemiological data -

In order to derive a relevant daily dose of oral arsenic exposure, the biomarker levels first needed to be converted into unadjusted urinary arsenic levels using the levels of creatinine (Cr) reported for the population cohort studied in the Strong Heart Study, 1.22 g/L of urine (Navas-Acien et al., 2009). A number of epidemiological studies found a near-perfect correlation between the levels of arsenic in drinking water and the levels in urine of individuals drinking the water, when

there was greater than 100 ppb arsenic. For example, a 97% correlation between water arsenic levels and the urinary arsenic levels was reported for a study cohort in Chile (Smith et al., 2009). The relationship becomes non-linear at lower water arsenic levels when the individual is consuming arsenic in food (Kile et al, 2007); however it was assumed that urinary levels would directly reflect intake. LogLogistic model was adjudged to be the best fit (as described in the following section) and the corresponding BMD_1 value (19.74 µg/g Cr) was selected as the representative BMD_1. The equivalent BMD for oral arsenic exposure was calculated as:

BMD_1 (µg/l)= [19.74 (µg/ g Cr) * 1.22 (g Cr/ l) / 0.97]

= 24.83 (µg/L)

Mouse data –

The $BMD_{1.0SD}$ generated by the BMD software for model Exponential 4 was used. This BMD value from mouse study was based on a low to moderate daily dose of oral arsenic exposure (ranging from 10 ppb to 200 ppb).

$BMD_{1.0SD}$= 36.61 (µg/L)

Conversion factor for mouse data to human equivalent BMD value –

An average adult lab mouse weighs approximately 25 gms and consumes 3.7 mL water per day. Hence per unit body weight the water consumption for a lab mouse is 0.148L/kg/ day. As a comparison, an adult human being drinks 2L water per day and has an average weight of 60 kgs or 0.033 L/kg/ day consumption of water per unit body weight. Thus a mouse drinks (4.48) times more water per unit body weight. This conversion factor would be different if the average body

weight for an adult is assumed to be 70 kg or 80 kg. The consumption rate of water per unit body weight would be (0.029 L/ kg/ day) and (0.025 L/ kg/ day) respectively. These would then denote that per unit body weight, a lab mouse drinks (5.1) or (5.92) times more water than a human being.

5.3.2 Reference dose derivation - Including application of Uncertainty Factors (UFs)

Consideration of the available dose-response data for CHD incidence and clinical endpoint etiology (e.g., atherosclerosis) led to the selection of the epidemiological study (Moon et al., 2013) along with data from mouse studies (primary data from our collaborator Dr. Koren Mann that was analyzed for BMD analysis) as the principal studies for derivation of RfD. Based on epidemiological data, BMD_1 of 19.0546 (μg/g Cr) (LogLogistic model) was selected as the POD for derivation of the chronic RfD. Another RfD was derived from mouse data by using $BMD_{1.0SD}$ of 36.6051 ppb (Exponential 4 model) as the POD.

The general formula for calculating the RfD was:

RfD = [BMD (μg/L)*(daily water consumption)] ÷ (Average body weight) ÷ Uncertainty Factors)

The EPA addresses how to derive a reference dose in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), which defines uncertainty factors of concern. The guidelines address 5 areas of uncertainty that can reduce the observed BMD or reference dose where there is no risk of disease by several orders of magnitude.

Uncertainty factors are divided into the reference dose to provide a margin of safety depending on the intensity of uncertainty the values of factors can be as low as 1 (no uncertainty), 3 (intermediate uncertainty) or 10 (high level of uncertainty).

Uncertainty Factor Consideration for Epidemiological data

A composite UF of 10 was applied to the selected POD to derive an RfD based on application of BMD analysis to epidemiological data.

- An intra-species uncertainty factor of 10 was applied. This was due to accounting for high levels of variation in terms of cardiovascular disease development within human populations exposed to arsenic. The concern was that the Native American population analyzed in the SHS might not reflect the US population as a whole. In addition, factors such as sex, age, body mass index and smoking status account for variability of response to oral arsenic exposure in human populations.
- An inter-species uncertainty factor of 1 was applied. This uncertainty factor accounts for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between animals and humans following exposure to the toxicant in consideration. Use of epidemiological data renders this uncertainty factor inapplicable in the RfD derivation.
- A database uncertainty factor (meant to account for database deficiency) of 1 was used. Since Moon et al. (2013) is a prospective cohort study that had a long and thorough follow-up period of about 20 years, the data used for POD analysis is considered adequate.
- A sub-chronic to chronic uncertainty factor of 1 was used. This uncertainty factor is needed to account for extrapolation of data from a sub-chronic exposure to a chronic exposure. Since the follow up period in the cohort studied by Moon et al., (2013) is 20 years, this study was

considered a chronic exposure study. An exposure can be defined as chronic if it lasts longer than approximately 10% of an individual's average life span.

- A LOAEL to NOAEL uncertainty factor of 1 was used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of 10% extra risk of mortality (due to arsenic induced coronary heart disease) was considered to be a minimally biologically significant level of effect. This BMR reflects the severity of the critical effect.

Uncertainty Factor Consideration for mouse data

A composite UF of 30 was applied to the selected POD to derive an RfD based on BMD analysis of mouse data.

- An intra-species uncertainty factor of 10 was applied. The mouse data is derived from study conducted with ApoE null mice that are genetically susceptible to develop atherosclerotic lesions. The ApoE null mice may not represent the response of other genetic variants of mouse species to arsenic exposure in terms of developing atherosclerotic plaques.
- An inter-species uncertainty factor of 1 was applied. Mice are considered very close to humans in toxicokinetic and toxicodynamic parameters with respect to cardiovascular disease (Straub et al., 2008; States et al., 2009) and the $ApoE^{-/-}$ mice responded to arsenite in a human relevant dose range. Therefore this factor was considered irrelevant for contributing to uncertainty towards calculation of RfD.
- A database uncertainty factor (meant to account for database deficiency) of 1 was used because the animal study covered a broad spectrum of doses and was conducted for a long span of 13 weeks.

- A sub-chronic to chronic uncertainty factor of 3 was used. Lab mice have an average life span of 2-3 years. Study period extending to 13 weeks may therefore be considered to be a chronic exposure. In order to account for variability in the life spans of different mice variants, an UF of 3 was applied in this case.
- A LOAEL to NOAEL uncertainty factor of 1 was used. In this case, a BMR level of one control SD was used to derive BMD.

5.4 RESULTS

No biologically-based models are available for arsenic induced atherosclerosis leading to coronary heart disease in humans. In this situation, as per EPA's practice (USEPA n-Butanol, 2011), a range of models was evaluated to determine the best way to empirically model the dose-response relationship in the range of the observed data. For mouse data, all models that are considered as part of USEPA's BMDS for analysis of continuous data were considered to be consistent with biological processes. The Hill model was an exception as it is considered to apply only when the biological response has been established to be receptor mediated.

All available dichotomous models in the EPA's BMDS (version 2.4) were fit to the epidemiological datasets for the increased incidences of CHD. All the tested models except Weibull and Gamma were considered biologically consistent. Tables 16, 17 and 18 summarize the BMD modeling results for these two datasets.

Goodness of Fit test statistics

LogLogistic model was considered to be the best fitting model for the CHD data based on the criterion for the lowest AIC value, adequate fit (p-value > 0.1) and BMDL estimates being in a range of two to three - folds (Table 16). For the continuous data from mouse studies, all models except models Exponential 2 and Exponential 3 fit the data well based upon the *p*- values being greater than 0.1 (Table 17).

Table 16 - BMD modeling based on CHD incidence data from Moon et al. (2013): Summary data for the fits of the models

Model	AIC	*p*- value	BMD_1 (μg/ g Cr)	$BMDL_1$ (μg/ g Cr)
Logistic	3911.17	0.35	3.52	2.23
LogLogistic	3909.32	0.88	19.74	2.24
LogProbit	3911.32	0.62	19.05	0.67
Probit	3911.19	0.35	3.47	2.17
Multistage	3910.36	0.53	8.87	2.001
Quantal Linear	3911.31	0.33	3.21	1.83
Weibull	Error^			
Gamma	Error^			

^ *Gamma and Weibull plotters generated error reports as the number of parameters being estimated were greater than the number of dose groups in the data*.

The Exponential 2 and 3 models were not considered for further evaluation due to unacceptable *p*-values. Based on the criterion for the lowest AIC value, Exponential 4 and Exponential 5 appear to fit the data equally well.

Table 17 - BMD modeling based on continuous data on surface area of atherosclerotic lesions in mice exposed to arsenic in drinking water: Summary data for the fits of the models

Model Name	*p*-value	AIC
Exponential2	0.05	106.42
Exponential3	0.05	106.42
Exponential4	0.44	102.35
Exponential5	0.44	102.35
Hill	Not valid*	
Linear	0.65	104.71
Polynomial	0.44	104.71
Power	0.65	104.71

***** *Hill model is consistent with the receptor-mediated response. It is not valid in this case, as the mechanism of arsenic induced atherosclerotic lesions is not receptor-mediated.*

Table 18 - Observed and predicted responses for best - fit models considered: Exponential 4 and 5

		Response Means			Response Standard Deviations			Scaled Residuals	
Dose	**N**	**Observed**	**Exp 4**	**Exp 5**	**Observed**	**Exp 4**	**Exp 5**	**Exp 4**	**Exp 5**
0	8	0.4625	1.07	1.07	0.9054	2.65	2.65	-0.645	-0.645
10	5	3.05	1.79	1.79	1.21	2.65	2.65	1.06	1.06
50	5	4.42	4.685	4.685	2.69	2.65	2.65	-0.22	-0.22
100	6	8.25	8.303	8.303	1.475	2.65	2.65	-0.05	-0.05
200	8	15.56	15.54	15.54	4.81	2.65	2.65	0.02	0.023

The scaled residuals for Exponential 4 and Exponential 5 are acceptably small (<1.0) at all doses except 10 ppb. Comparing the values of scaled residuals of interest provides an estimate of the scaled difference between the observed and predicted means for the dose group that is closest to the calculated BMD. Exponential 4 and Exponential 5 models were selected for BMD estimation (Table 18) based on the above considerations. However, a formal Chi-squared test of the differences between the Exponential 4 and Exponential 5 likelihoods shows that one would not reject the hypothesis that Exponential 4 and Exponential 5 provide equivalent fits (p = 1.641). Exponential 4 was therefore chosen as the simplest model that gives a reasonable fit to the data.

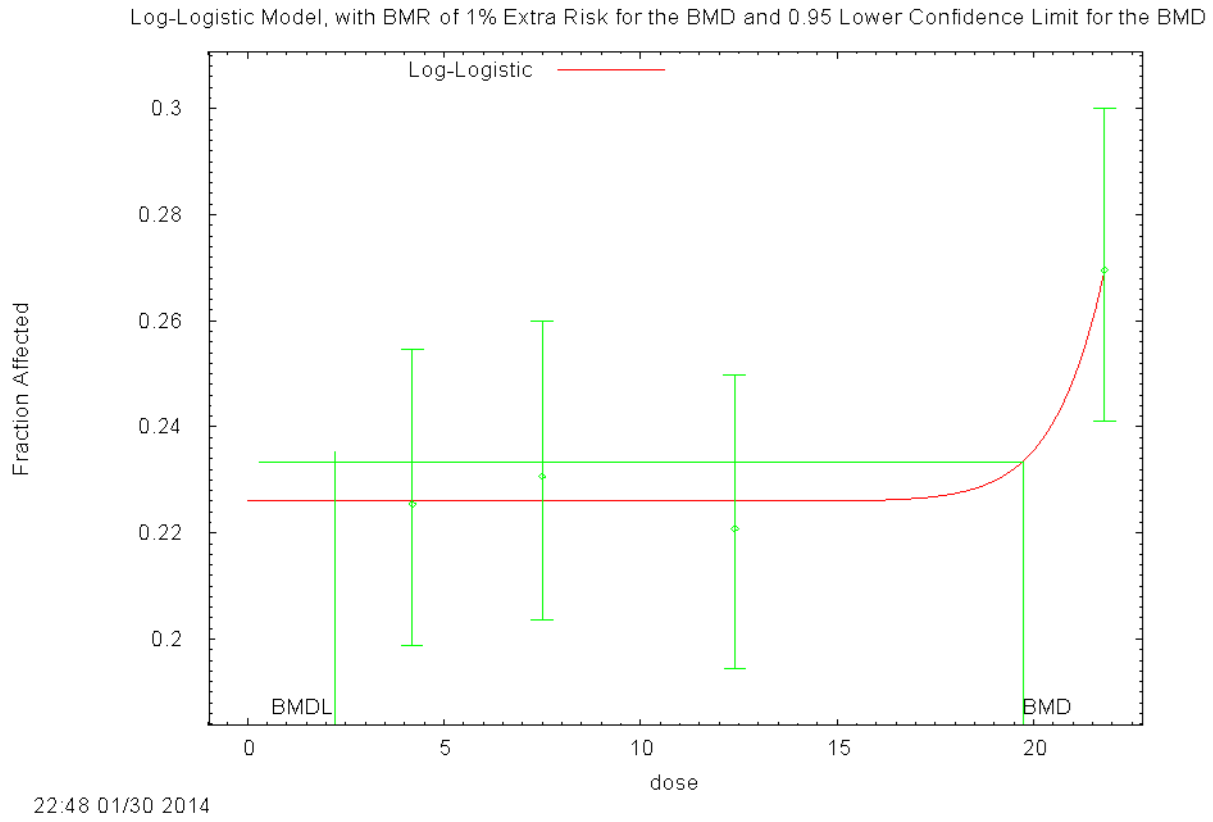


Figure 11 - BMD analysis for epidemiological data: LogLogistic model with BMR of 1% extra risk for BMD

X - axis represents the dose in µg/g creatinine of urinary arsenic levels. Y - axis shows the fraction of exposed population affected with CHD.

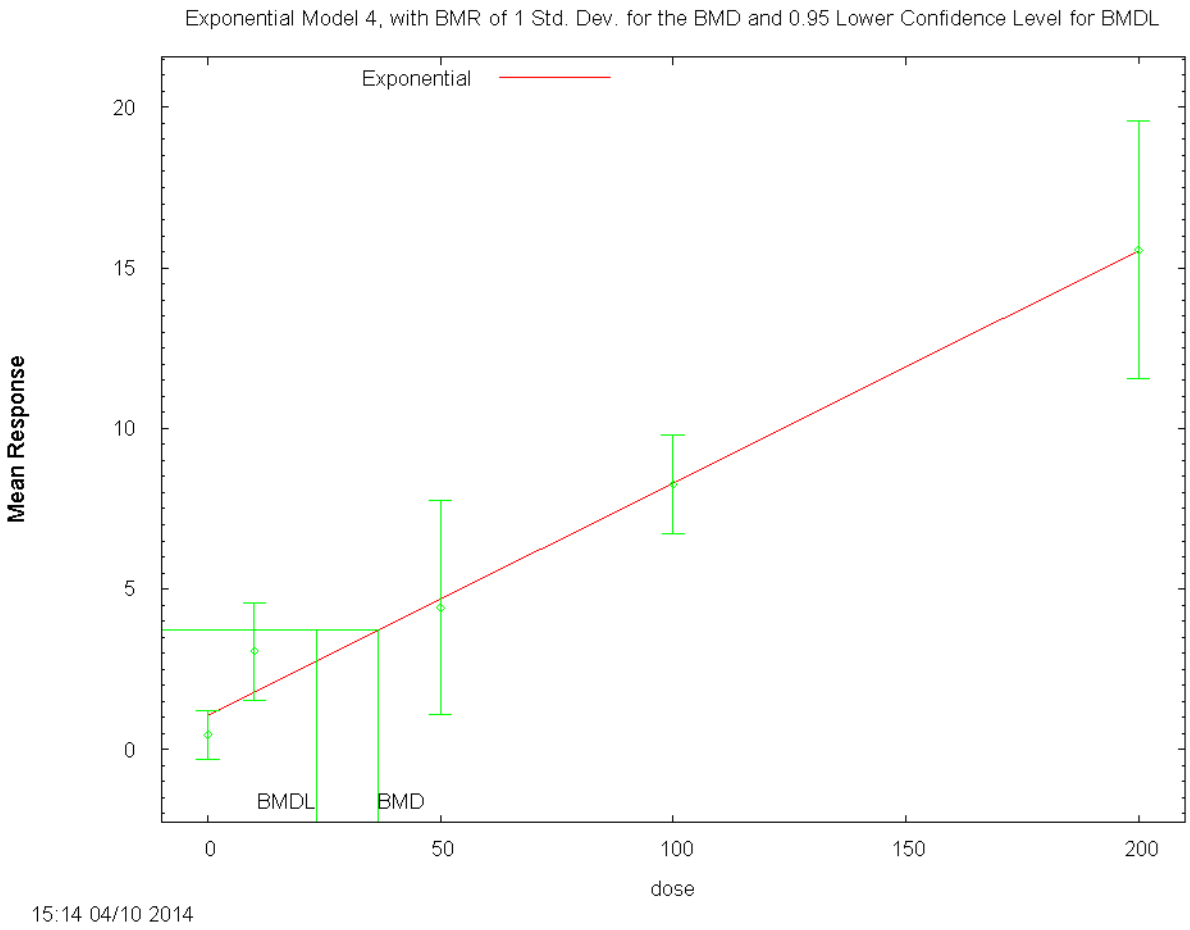


Figure 12 - BMD analysis for mouse data: Exponential 4 model with BMR of 1.0 standard deviation extra risk for BMD

X- axis represents the dose in µg/L of arsenic in drinking water. Y - axis shows the fraction of area of the aortic arch with atherosclerotic lesions

Computation of BMD

Epidemiological data -

BMD_1 value from the best fitting model (LogLogistic) was based on biomarker data of urinary levels of arsenic adjusted for creatinine.

BMD_1= 19.74 (μg/g creatinine)

Equivalent BMD for oral arsenic exposure (as described in the Methods section)

= 24.83 (μg/L)

Mouse data:

$BMD_{1.0SD}$ for Exponential 4 model was adjudged best- fit based on the comparison of all goodness of fit parameters as described in the previous section.

$BMD_{1.0SD}$ = 36.61 μg/L

Reference Dose Derivation —Including Application of Uncertainty Factors (UFs)

RfD based on epidemiological data (Moon et al., 2013)

Consideration of uncertainty in the human epidemiological data yielded a composite UF of 10 as described in the section 5.3.2. Applying the BMD_1 value for epidemiological data, RfD for oral arsenic exposure for an average adult consuming 2L water per day, was calculated as follows

RfD = 24.83 (μg/L) * [2 L/day] ÷ Average adult body weight ÷ 10

Assuming average adult body weights of 60 kg (Walpole et al, 2012), 70 kg or 80 kg to account

for variability, the RfD (μg/kg/day) values are presented in Table 19.

RfD based on animal data

Uncertainty factors, addressing five areas of uncertainty resulting in a composite UF of 30, were applied to the selected POD to derive an RfD based on application of atherosclerotic lesion data in mice. Applying the $BMD_{1.0SD}$ value for mouse data, and the conversion factor for mouse data to human data (as described in section 5.3.1), RfD for oral arsenic exposure for an average adult human was calculated as follows:

RfD = [36.61 μg/L * 3.7 mL÷ Average body weight for lab mouse ÷ UF] ÷ conversion factor

= (0.18 μg/kg/day) ÷ conversion factor

Table 19 presents the different RfD values assuming average adult human body weights of 60 kg (Walpole et al., 2012), 70 kg or 80 kg to account for variability.

Table 19 - Estimated RfD values for oral arsenic exposures based on epidemiological data and mouse data

RfD (μg/kg/day)	**Average adult body weight 60 kg**	**Average adult body weight 70 kg**	**Average adult body weight 80 kg**
RfD (μg/kg/day)[a]	0.083	0.071	0.063

RfD (μg/kg/day)[b]	0.04	0.035	0.03

[a] RfD based on Moon et al., 2013 epidemiological data

[b] RfD based on primary data from mouse studies

Calculating risk and a global burden of CHD from foodborne arsenic

The data from dose–response and exposure assessments were integrated to quantify the burden of arsenic-related cancers across the world to characterize the risk of coronary heart disease due to foodborne arsenic. Table 9 (Chapter 4) provides the estimated range of dietary exposure to inorganic exposure for all the 13 GEMS clusters. The estimated range of daily dietary inorganic arsenic exposure was divided by the BMD_1 value based on epidemiological data. This risk was then multiplied with the background rate of coronary heart disease in that region/country to estimate the increase in total number of CHD cases per year that can be attributed to dietary inorganic arsenic.

As an illustration, the following are the estimates for USA. Based on similarities of dietary pattern, WHO GEMS 13 cluster approach (WHO GEMS, 2006) classifies USA under GEMS Cluster M. For this cluster, Table 9 provides 6.44 to 70.56 μg/day as the range of dietary inorganic arsenic exposure. It should be mentioned that the lower bound of this range (6.44 μg/day) is highly conservative as it represents the lower estimate of total arsenic content in different components of the diet, as well as the lower range of inorganic content of the different

food categories and the lower end of the bioavailability (50%). The upper bound of arsenic exposure is based on the scenario that all the components of diet have the maximum detected total arsenic content; highest possible fraction of inorganic arsenic and the inorganic arsenic is 100% bioavailable. All these factors contribute to the wide range of dietary inorganic arsenic exposure estimates for this cluster.

Thus to calculate the potential burden of CHD from the highest exposure to arsenic in GEMS cluster M:

(Upper bound estimate of bioavailable iAs in food) (µg/day)/BMD (µg/day)

= (70.56 (µg/day)/(49.66 (µg/day) = 1.42

Similarly, the potential burden of CHD from the lowest exposure to arsenic in the cluster would be:

(Lower bound estimate of bioavailable iAs in food) (µg/day)/BMD (µg/day)

= (6.44 (µg/day)/(49.66 (µg/day) = 0.13

USA was selected as a representative country for this GEMS cluster M. With regard to incidence, data from the Atherosclerosis Risk in Communities (ARIC) and Cardiovascular Health Study in USA indicate that annually, 785,000 new coronary attacks occur (DeBacker, 2009). The following algorithm was used to calculate the burden of disease due to the upper bound of arsenic in food:

Risk * CHD incidence rate

=1.42*785,000 = 1,114,700

This gives the worst-case scenario number of 329,750 (calculated incidence – back ground incidence) extra CHD cases annually attributable to foodborne arsenic exposure for a total population of 313.9 million in USA that assumes everyone in the USA ate the same diet every day. Conversely, using the risk value for the lower bound of exposure in GEMS cluster M, the hazard quotient falls well below 1.0 indicating that there is no increase in attributable CHD disease with consumption at the lower bound. As seen in Table 21, consuming food with the lowest bound of available inorganic arsenic content poses no increased CHD burden in all of the clusters. However, the risk from consuming food at the upper bound is significant in all clusters except clusters A and I.

Table 20 - Risk characterization for coronary heart disease corresponding to foodborne inorganic arsenic exposure at 50 - 100% bioavailability in 13 WHO-GEMS clusters of countries (adapted from Table 9)

GEMS Cluster	Lowest boundary of iAs (50% bioavailable) (µg/day)^	Upper boundary of iAs (100% bioavailable) (µg/ day)^	Lower bound estimate for risk characterized for CHD incidence	Upper bound estimate for risk characterized for CHD incidence
A	4.8	53.4	0.10	1.07
B	10.37	108.35	0.21	2.18
C	9.09	85.46	0.18	1.72
D	6.71	66.95	0.14	1.35
E	5.75	63.45	0.12	1.28
F	5.25	57.27	0.11	1.15
G	7.82	75.14	0.16	1.51
H	6.44	66.54	0.13	1.34
I	5.02	52.2	0.10	1.05
J	5.01	51.88	0.10	1.04
K	6.6	66.13	0.10	1.33
L	7.88	79.1	0.16	1.6
M	6.44	70.56	0.13	1.42

Similarly for United Kingdom, which falls in Cluster E, the upper bound estimate for risk characterized for CHD incidence is 1.28. The CHD statistics of the British Health Foundation (Scarborough et al., 2010) indicate that the average annual incidence rate of myocardial infarction is 800 per 200,000. For the total population of the UK in 2010 (62.3 million), the derived incidence would be 249, 200. Using the same algorithm, the burden of disease due to the upper bound of arsenic in food in the United Kingdom was

Risk* CHD incidence rate

= 1.28* 249, 200

= 318,976

Thus, for United Kingdom in the worst-case scenario 69, 776 (calculated incidence – back ground incidence) extra CHD cases per year can be attributed to foodborne arsenic exposure. Assuming that in the UK regardless of age, and sex, everybody ate essentially the same diet, arsenic in food can cause an increase of 28% in CHD incidence over the background rate. While in USA, it can cause upto a 42% increase in incidence over the background. However this increase accounts to only about 0.1% of the total populations of the two countries individually, indicating that even in the worst case scenario, the risk is significant but not overwhelming.

5.5 DISCUSSION

Using quantitative risk assessment approach, the reference dose for foodborne inorganic arsenic induced CHD was derived. This research used BMD analysis to conduct dose-response

assessment for coronary heart disease, a major non-cancer disease endpoint due to arsenic exposure (NRC, 2013). BMD analysis is well suited for risk assessments based on dichotomous, as well as continuous data, from exposure studies, although it is often difficult to identify discrete dose-response thresholds in the latter.

USEPA BMD software does not have the ability to factor in adjustments made for confounders like age, sex, smoking and other socio-demographic factors that are epidemiologically significant. Therefore, primary data from mouse studies was analyzed and applied to BMD modeling to provide a comparison to the BMD values generated from epidemiological data. In this analysis, the RfD was calculated using BMD values instead of BMDL. BMDL is the statistical lower confidence limit on the dose or concentration at the BMD, and provides a conservative estimate of the dose that can cause a predetermined increase in adverse effect. Thus it is conservative to default to BMD instead of BMDL for estimating a safe dose of arsenic in food. Also, the estimated BMD for 1% BMR is very close to the observed significant increase in the hazard ratios for coronary heart disease incidence reported in the Strong Heart Study (Moon et al., 2013). The BMD_1 value of 19.74μg/g Cr (urinary arsenic levels) derived here corresponds to the hazard ratio of 1.2 in a study cohort of 3575 individuals (Moon et al., 2013), corresponding to approximately 15% of the exposed participants for coronary heart disease incidence.

The estimated RfD derived from animal data is more conservative than the RfD for epidemiological data. This might be attributed to the fact that the animal data uses atherosclerotic lesions as the observed endpoint. Although atherosclerosis is the underlying pathology of most clinical manifestations of coronary heart disease, it is pre-clinical evidence and more people will

have plaques than will advance to CHD. The conversions used to derive equivalent BMD values for humans are based on inter-species conversions for amount of daily water consumption per unit body weight difference between mice and humans. Despite the progress that has been made toward prevention and cure of coronary heart disease, it appears that actual abilities are limited to a retardation of atherosclerosis and a postponement of CHD to older age (DeBacker, 2009). Given the demographic changes that are taking place in most communities, further increase in the absolute number of people with CHD is expected. Most of the available data on risk of CHD from arsenic are based on studies of high levels of arsenic in drinking water. Limited numbers of studies provide exposure information at low to moderate levels of arsenic exposure. Another limitation in analysis of burden of CHD is that most of the studies and databases report the mortality data for CHD on a country/ WHO region level. This makes it difficult to translate the estimate of risk to annual additional cases of CHD expected due to arsenic exposure through food. Also, the incidence of CHD is age-specific, but the estimates for dietary arsenic induced burden of CHD for USA presented here are an overall estimate inclusive of all age groups.

The research framework presented here enables assessment of the additional burden of coronary heart disease due to inorganic arsenic exposures through food. It should be noted that while the estimates for USA represent the additional disease burden, this number does not reflect additional risk for entire US population. This is because the upper bound estimate of foodborne inorganic arsenic that is bioavailable is realistic for only a small portion of the US population whose entire diet corresponds to high arsenic content. The possible dissimilarity in diets is a major limitation of using the WHO-GEMS dietary cluster approach. GEMS cluster approach works on the major assumption that the individuals within each GEMS cluster consume roughly comparable amounts of the foodstuffs that are presented in the GEMS database. This applies

across age groups and genders. On the other hand, if the exposure from water is also brought under consideration, then the risk of arsenic induced coronary heart disease would be somewhat higher than these estimates.

The expected burden of disease attributable to foodborne arsenic is a function of the baseline risk to CHD in the population being studied. So any additional estimates are influenced by the pre-existing incidence of CHD in that population. The estimated burden of disease attributable to foodborne arsenic exposure directly corresponds to the characterized risk values (Table 20) for CHD incidence. Additionally, the shape of the dose-response curve beyond the threshold would also affect the estimates of CHD incidence. It is a limitation that the current analysis does not allow the prediction of whether the dose response curve would be linear or non-linear beyond the threshold. Even with the assumption of linearity, it would not be possible to estimate the slope of the linear curve. However, this research is based on an assumption of linearity of the dose response curve beyond the threshold point with 1:1 correlation between the dose and response. The estimates presented in this research strongly indicate that exposure to food containing lower range of arsenic content is not responsible for any additional burden of CHD in any cluster or country worldwide. The CHD burden estimates for USA and UK presented in this research are within the range of observed increase in cardiovascular disease mortality (Chen et al, 2011). In this prospective cohort study of 11, 746 participants in Bangladesh, an increase of 22% was reported in the risk of mortality from cardiovascular for arsenic exposure levels of 12 ppb to 62 (Chen et al., 2011). The range of exposure estimates for bioavailable inorganic arsenic in food for USA and UK (Cluster M and E respectively) is comparable (between 5.75 to 70.56 μg/day) to the level of arsenic in drinking water associated with the level of observed increase in risk in Chen et al. (2011) (Table 9). It is also comparable to the ATSDR estimate of an average

consumption of 40 μg/day of arsenic across the USA (reference website). In addition, based on a duplicate diet survey in Bangladesh, Kile et al. (2007) observed that the background dietary total arsenic intake for the population, calculated using the dietary exposures for the participants with no detectable arsenic in their drinking water, was 46 μg/day. This indicates that based on our estimated BMD_1 value for epidemiological data, 49.66 μg/day, the entire study population in Bangladesh might be at a risk of developing CHD due to foodborne arsenic

The relationship between biomarkers (e.g., urinary and toenail levels) and low levels of drinking water arsenic concentrations was found to be non-linear but becomes linear as arsenic levels in drinking water increase (Karagas et al., 2000; Kile et al., 2005, 2007; Watanabe, 2001). It is therefore likely that the added exposure from dietary sources explains the observed non-linearity in these relationships. This non-linearity as well as a daily consumption of 2 liters of water for an average adult were taken into account while deriving an equivalent oral dose of arsenic exposure from the estimated BMD_1 based on creatinine adjusted urinary arsenic levels.

In conclusion, the results of this quantitative risk assessment indicate that similar to what was found for the incidence of bladder, lung and skin cancer, the consumption of arsenic in food can increase the burden of CHD. This research also estimates that the risk of CHD incidence is not increased at the low end of exposure to foodborne arsenic. It appears that this risk occurs globally only when consuming food with the upper bound of available inorganic arsenic levels and that consumption of foods containing the lower bound of arsenic does not increase the burden of CHD in any cluster country. All the limitations of exposure assessment, for the ranges of arsenic content in food as well as other risk modifying factors that were discussed in context of GBD for cancers, apply to these CHD burden estimates. The risk estimates are nonetheless

valuable for informing policies to reduce the global burden of disease from arsenic exposures in food. The main policy conclusion that can be drawn is that the global burden of CHD from foodborne arsenic might be reduced or eliminated if foods with the lower bound of arsenic content are consumed.

6.0 CONCLUSIONS

Multiple epidemiological studies indicate an association between arsenic exposure and an increased risk for bladder cancer, lung cancer and non-melanoma skin cancer (IARC, 2012). Cardiovascular disease may be the most important non-cancer disease risk posed by environmental arsenic exposures, given the high burden of this disease worldwide (NRC, 2013). The World Health Organization (WHO) Foodborne Disease Burden Epidemiology Reference Group (FERG) is focused on estimating the global burden of disease from foodborne chemical exposures, including dietary inorganic arsenic exposure. The data presented in this thesis demonstrate that inorganic arsenic in food causes a significant burden of cancer, as well as non-cancer diseases globally. Estimates of global disease burden enable policy makers to prioritize the allocation of limited resources to improve public health in the most effective manner. Reducing the daily consumption of food containing the highest levels of arsenic (e.g., certain cultivars of rice) may be the most effective means of reducing the global burden of disease caused by foodborne arsenic.

6.1 ESTIMATION OF GLOBAL FOODBORNE INORGANIC ARSENIC EXPOSURE

The level of inorganic arsenic in different food items was abstracted from literature sources including recent reports by Joint Expert Committee of Food Additives (JECFA, 2011) as well

European Food Safety Authority Report (EFSA, 2009). To estimate the total consumption of food worldwide, World Health Organization Global Environmental Monitoring Systems (WHO GEMS, 2006) databases were utilized. WHO divides all countries of the world into 13 clusters based on similarities in their dietary patterns. This analysis provided a range of dietary arsenic exposure where the lower and upper bounds respectively denote the lowest and highest levels of total bioavailable inorganic arsenic content. The GEMS cluster approach has certain limitations including the assumption that individuals within each GEMS cluster consume roughly comparable amounts of the foodstuffs that are presented in the GEMS database, including across age-groups and sex. Moreover, it contains an inherently broad range of dietary variation between the countries within each cluster (Liu et al., 2010). One of the major assumptions in the current analysis for estimating the inorganic arsenic content for different food groups is that the speciation and arsenic content of foods cultivated in different regions of the world would be the same. The GEMS cluster data also do not provide consumption details for certain miscellaneous food items (e.g., seaweed) with reportedly high levels of inorganic arsenic. However, GEMS cluster approach does provide a uniform platform for comparing the exposure estimates for foodborne arsenic. Most importantly, it highlights the impact of change in dietary patterns on exposure to foodborne toxicants like arsenic. For example, rice is a staple diet for certain populations in different parts of the world and different cultivars of rice tend to accumulate arsenic from the soil and irrigation water to different degrees. Monitoring the levels of arsenic in the soils and waters where the rice is grown or planting cultivars that have relatively lower uptake of arsenic would help to reduce the intake of inorganic arsenic.

6.2 GLOBAL BURDEN OF DISEASE FOR SKIN, LUNG AND BLADDER CANCER CAUSED BY ARSENIC IN FOOD

A literature review of epidemiological data associating oral arsenic exposure to bladder, lung and non-melanoma skin cancers in Chapter-3 provided a conclusive association of arsenic with these cancers. This research relied on linear extrapolation as the default conservative approach for dose-response assessment (USEPA, 2005) because there is insufficient information about the mode of action of arsenic-induced cancers. Although EPA IRIS also derived a single slope factor for arsenic-related skin cancer, the assumption of linear dose-response relationships of arsenic-related cancers is contentious (USEPA, 1998). As the majority of epidemiological evidence for arsenic induced disease is based on exposure to arsenic in water, it is reasonable to derive a slope factor based on water arsenic data from Taiwan (Morales et al., 2000). This was done by converting the water arsenic exposure to equivalent dietary arsenic exposures beforehand. The estimates presented here could not rule out the confounding of disease burden for lung cancer by smoking.

The global burden of disease estimates presented in this thesis are highest for all three cancer types in countries that fall in GEMS cluster G. This cluster comprises of the most densely populated countries of the world like China, India and Bangladesh and the GBD estimates reflect the large population size of these countries. Indeed, other recent studies have also reported rapidly rising cancer incidence and high cancer mortality rates in China and India contributing to a major portion of global cancer burden (Goss et al., 2014; Collingridge, 2014). In addition, cluster G countries geographically possess high arsenic content in the bedrock thus exposing the population to a high overall rate of chronic arsenic exposure through more than one route of

exposure. All these factors can potentially pre-dispose the population to develop arsenic induced cancers. However, the prevalence of arsenic in the soils and bedrock is not uniform throughout cluster G countries, nor are the dietary constituents (e.g., arsenic in rice) consistent across the entire population in the cluster. Thus the averaging from the GEMS cluster approach may over- and under-estimate the true risk of cancer in a given population within the cluster.

6.3 GLOBAL BURDEN OF CORONARY HEART DISEASE

A recent National Research Council report concluded that cardiovascular disease are the non-cancer diseases where incidence is most clearly correlated with environmental arsenic exposures (NRC, 2013). A literature review of epidemiological studies on arsenic related cardiovascular effects was conducted with a special emphasis on CHD. The primary pathologic process that underlies cardiovascular disease is atherosclerosis and it is clinically manifested as CHD, stroke, or peripheral arterial disease. This research applies BMD analysis approach as per USEPA BMDS guidelines and USEPA BMD software (version 2.4) to derive a RfD for foodborne arsenic induced CHD. The literature review identified the Strong Heart epidemiological study, as the best data set to carry into the BMD analysis (Moon et al., 2013). In this recent prospective cohort study, Moon et al., (2013) provided dose-response relationships for CHD incidence and mortality in a population exposed to relatively low levels of arsenic. The rigorous measure of clinical CHD endpoints combined with defining the low end of the dose response curve combined to demonstrate the value of using this data set to estimate disease risks from the relatively low amount of arsenic found in food. Other datasets from large prospective studies in cohorts exposed to high drinking water levels of arsenic often failed to account for disease at

lower exposure levels resulting in extrapolation of the dose-response relationship that was greatly biased by the high exposure levels.

USEPA BMD software is not equipped to handle adjustments made for epidemiologically significant confounders of age, sex, smoking and other socio-demographic factors. The Strong Heart study provided different models of the dose response relationship by eliminating the influence of many the confounders that could influence CHD in the unique Native American cohort. The final model eliminated effects of age, sex, smoking, diabetes, obesity and renal function. In addition to using this population based model, the RfD was derived based on the BMD analysis of primary mouse data for atherosclerosis, a model system where the intake of arsenic was well-controlled and the only confounding variable was the genetic predisposition of the mice to atherosclerosis. Although atherosclerosis is not the final clinical manifestation of arsenic-induced disease, it is the primary pathogenic process that evidently leads to CHD. It was remarkable that deriving the BMD and RfD from the two different data sets yielded values that were similar and within one or two fold of each other. This suggests that the cardiovascular impacts of arsenic are nearly identical between the species as opposed to the observation that mice are a poor model for arsenic-induced cancers.

The burden of CHD due to arsenic was estimated using BMD values from epidemiological data. The Rfd was not used for the determination, since it is the dose at which there is no risk of disease made more conservative by dividing by the uncertainty of the estimate to account for susceptibility in the population. The BMD reflects the true point of departure where disease occurs and thus a dose that would produce a burden of disease. It is important to emphasize that the BMD modeling produced an estimated point of departure that is almost identical to that

observed for the exposure of arsenic that posed a significant CHD hazard ratio in the dose response analysis in the Strong Heart Study (Moon et al., 2013). This burden of disease estimation quantifies the additional cases of CHD annually over the background incidence rate. The estimation however gets restricted by lack of incidence data for CHD in countries and WHO regions. Usual disease rates are reported as mortality which is not useful for the current estimation. Mortality data do not provide for estimation of morbidity and DALY. Therefore the current estimation relies on data from representative countries in two GEMS country clusters M and G. The current BMD estimates provide a conservative estimate, as these are based on maximum possible levels of inorganic arsenic in food that is completely bioavailable to cause disease.

6.4 PUBLIC HEALTH SIGNIFICANCE

This study answers an important public health question regarding global burden of cancer and non-cancer disease due to inorganic arsenic in food. Estimating the global cancer and non-cancer burden caused by foodborne arsenic exposure will support policies that reduce exposure to disease promoting environmental hazards. Arsenic induced cancers and cardiovascular disease have a considerable detrimental effect on global health. The additional annual cases attributable to foodborne arsenic exposure are a significant impact on the status of global health.

An accurate estimation of the extent of foodborne illness is expected to help ensure public health security as well as enable socioeconomic development worldwide (Kuchenmuller et al., 2009). Estimation of disease burdens like those presented in this thesis, allow evaluation of the current

food safety measures and development of new food safety standards. Policy makers can prioritize the allocation of limited resources to improve public health in the most efficient and effective manner. The knowledge of the burden of disease attributable to food toxicants helps to quantify the disease burden in monetary costs. The results demonstrate that a cost-effective strategy for reducing disease burden from arsenic in food is to reduce the consumption of cultivars that accumulate arsenic in the food. Simple shift in types of foods or amounts of contaminated food consumed, as well as providing alternative cultivars with low arsenic content, would be cost effective means of reducing disease burden.

6.5 SUMMARY AND FUTURE DIRECTIONS

Exposure to arsenic through food is a recent concern, although it has occurred as long as man has consumed crops. The scientific community is in the process of determining if exposure to arsenic via food raises the same health issues as exposure to arsenic through drinking water. Even if human biomarkers for arsenic exposure are available in urine, blood, hair and nails (JECFA, 2011), it is not possible to determine from these biomarker measurements what portion of arsenic exposure comes from drinking water and what portion comes from food. For the purposes of estimating human health consequences associated with arsenic, knowing the overall population arsenic exposure matters more than knowing the relative contribution from water vs. food; but for the purpose of recommending interventions, it can be helpful to understand the separate contributions. There are uncertainties surrounding the proportions of organic vs. inorganic arsenic for different food commodities. The inorganic arsenic is of greater toxicological importance. Bioavailability of arsenic in different foods tends to vary with the food group or the

method of processing of the food. Bioavailability is also influenced by the complexity of other food constituents. There is a need for more assessment of the disease risk from the lower end of arsenic exposures. Exposure estimates are weakened by difficulty obtaining accurate foodborne arsenic exposure data worldwide.

More research is needed to acquire an understanding of whether inorganic arsenic in food has the same toxicological effects, at the same doses, as inorganic arsenic in drinking water.

Knowledge of the interactions of arsenic with other dietary and environmental risk factors in inducing adverse health effects will help to provide more accurate estimates of global disease burden due to foodborne arsenic.

APPENDIX A. TSENG (1977): TABLE 1. AGE-SPECIFIC AND SEX-SPECIFIC PREVALENCE RATE FOR SKIN CANCER (ADAPTED)

Table A-1 - Tseng (1977): Table 1. Age-specific and Sex-specific Prevalence Rate for Skin Cancer (Adapted)

Age	Males		Females	
	Per 1000	**Number**	**Per 1000**	**Number**
0-19	-	0	-	0
20-29	1.0	2	1.1	3
30-39	9.7	20	1.5	4
40-49	25.9	40	8	16
50-59	80.8	99	28.9	38
60-69	124.8	92	57.0	40
70+	209.6	57	53.8	17
Total	16.1	310	5.6	118

APPENDIX B. MORALES ET AL. (2000): TABLE 2. COMPARISON POPULATION DATA

Table B-1 - Morales et al. (2000): Table 2. Comparison Population Data

Sex, age (years)	All- Taiwan			Southwestern Taiwan		
	PYR	Deaths (n)		PYR	Deaths (n)	
		Bladder cancer	Lung cancer		Bladder cancer	Lung cancer
Male						
20-25	13,271,386	3	45	2,956,638	2	14
25-30	11,054,191	4	86	2,175,046	3	26
30-35	8,628,516	8	144	1,580,019	2	33
35-40	6,793,545	20	217	1,320,637	6	38
40-45	6,375,466	50	447	1,327,866	18	89
45-50	6,384,052	91	951	1,334,769	34	181
50-55	6,062,515	164	1,852	1,214,443	52	323
55-60	5,018,542	213	2,882	977,820	61	478
60-65	3,666,535	345	3,557	739,460	103	595
65-70	2,443,367	413	3,569	520,965	126	607
70-75	1,480,126	418	2,658	320,158	130	465

75-80	720,375	305	1,318	158,750	88	230
80-85	287,294	146	512	63,236	32	80
85+	105,411	66	152	22,651	15	22
Female						
20-25	12,612,276	0	39	2,595,529	0	7
25-30	10,548,089	2	70	1,846,189	2	19
30-35	8,210,507	2	102	1,402,764	0	17
35-40	6,458,620	5	205	1,215,899	2	41
40-45	5,802,856	20	365	1,191,615	8	75
45-50	5,157,821	41	525	1,111,810	14	112
50-55	4,335,755	76	730	957,985	36	160
55-60	3,517,193	124	1,018	774,836	52	200
60-65	2,776,622	153	1,224	634,758	77	258
65-70	2,106,715	173	1,280	492,203	68	230
70-75	1,490,659	185	1,062	342,767	70	190
75-80	888,468	157	707	199,630	43	108
80-85	433,245	81	330	96,293	21	45
85+	217,590	41	136	46,089	9	10

APPENDIX C. NATIONS IN EACH OF THE 13 WHO GEMS CLUSTERS (WHO GEMS, 2006)

Table C-1a - Nations in each of the WHO GEMS clusters A through D (WHO GEMS, 2006)

Cluster A	Cluster B	Cluster C	Cluster D
Angola	Cyprus	Algeria	Albania
Burundi	Greece	Egypt	Armenia
Cameroon	Israel	Iraq	Azerbaijan Belarus
Central African Republic	Italy	Jordan	Bosnia and Herzegovina
Comoros	Lebanon	Kuwait	Bulgaria
Côte d'Ivoire	Portugal	Libya Arab Jamahiriya	Georgia
Djibouti	Spain	Morocco	Iran, Islamic Rep. of
Eritrea	Turkey	Saudi Arabia	Kazakhstan
Ethiopia	United Arab Emirates	Syrian Arab Republic	Kyrgyzstan
Gabon		Tunisia	Moldova, Republic of
Guinea			Montenegro
Guinea Bissau			Romania
Liberia Madagascar			Russian Federation
Mauritius			Serbia Tajikistan
Rwanda			The former Yugoslav

Sao Tome & Principe			Republic of
Seychelles			Macedonia
Sierra Leone			Turkmenistan
Somalia			Ukraine Uzbekistan
Uganda			
Yemen			

Table C-1b - Nations in each of the WHO GEMS clusters E through H (WHO GEMS, 2006)

Cluster E	**Cluster F**	**Cluster G**	**Cluster H**
Austria	Estonia	Afghanistan	Bolivia
Belgium	Finland	Bangladesh	El Salvador
Croatia	Iceland	Cambodia	Guatemala
Czech Republic Denmark	Latvia	China	Haiti
France	Lithuania	India	Honduras
Germany	Norway	Indonesia	Mexico
Hungary	Sweden	Lao People's	Nicaragua
Ireland		Democratic Republic	Panama
Luxembourg		Malaysia	Paraguay
Malta		Mongolia	Peru
Netherlands		Myanmar	Saint Kitts & Nevis
Poland		Nepal	Saint Vincent & the

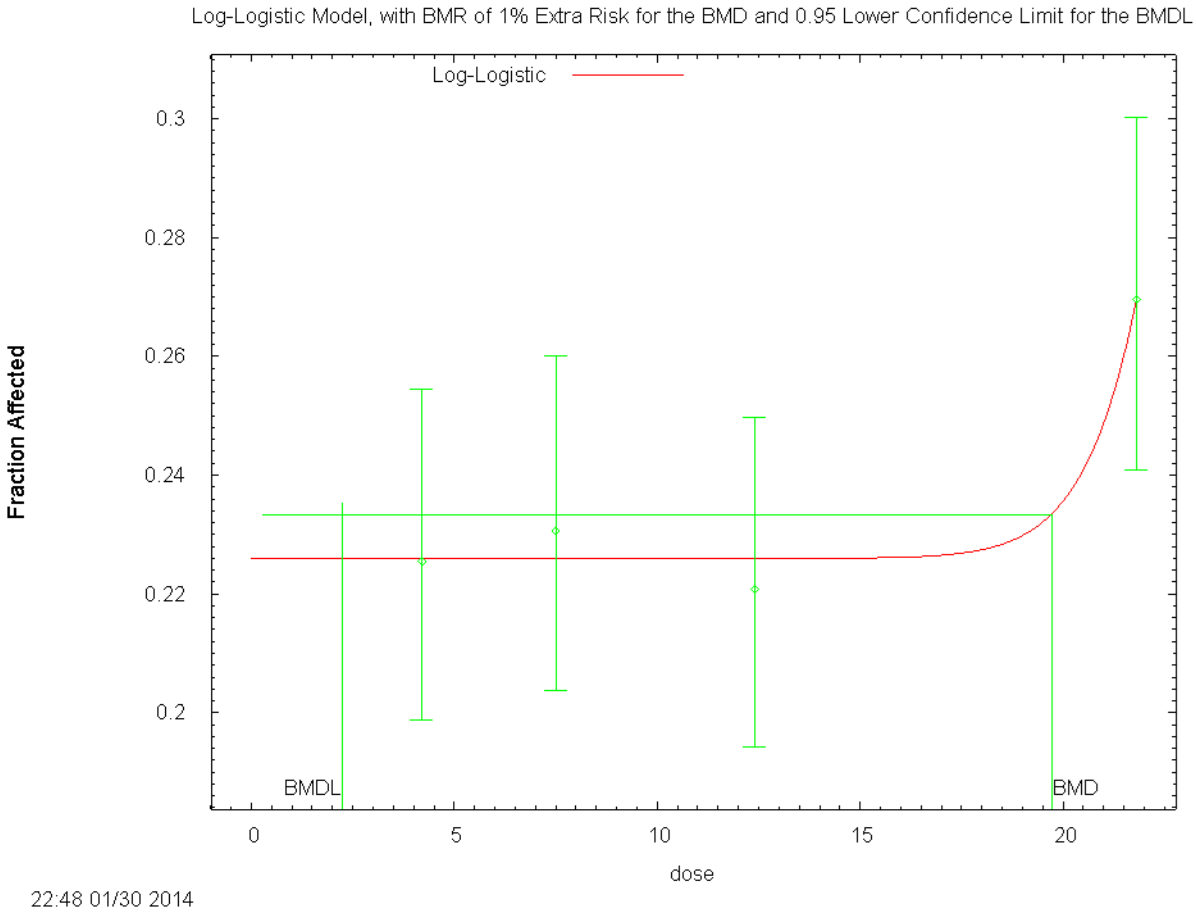
Slovakia Slovenia Switzerland United Kingdom of Great Britain and Northern Ireland		Pakistan Sri Lanka Thailand Vietnam	Grenadines

Table C-1c - Nations in each of the WHO GEMS clusters A to DI through M (WHO GEMS, 2006)

Cluster I	**Cluster J**	**Cluster K**	**Cluster L**	**Cluster M**
Benin Botswana Cape Verde Ghana Kenya Lesotho Malawi Mozambique Namibia South Africa Swaziland Togo	Burkina Faso Chad Congo, Democratic Republic of Congo Gambia Mali Mauritania Niger Nigeria Senegal Sudan	Antigua & Barbuda Bahamas Barbados Belize Bermuda Brazil Colombia Costa Rica Cuba Dominica Dominican Republic	Brunei Darussalam Fiji French Polynesia Japan Kiribati Korea (Democratic People's Republic of) Korea (Republic of) Maldives New Caledonia Papua New Guinea Philippines	Argentina Australia Canada Chile New Zealand United States of America Uruguay

United Republic of Tanzania Zambia Zimbabwe		Ecuador Grenada Guyana Jamaica Netherlands Antilles Saint Lucia Suriname Trinidad and Tobago Venezuela (Bolivarian Republic of)	Solomon Islands Vanuatu	

APPENDIX D. USEPA BMD SOFTWARE OUTPUT FOR EPIDEMIOLOGICAL DATA (MOON ET AL., 2013) FOR LOGLOGISTIC MODEL (BEST-FIT)



==========

Logistic Model. (Version: 2.14; Date: 2/28/2013)

Input Data File: C:/Shilpi/BMDS240/Data/lnl_Dax_Setting.(d)

Gnuplot Plotting File: C:/Shilpi/BMDS240/Data/lnl_Dax_Setting.plt

Thu Jan 30 22:48:04 2014

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BMDS_Model_Run

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The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = Effect

Independent variable = Dose

Slope parameter is restricted as slope >= 1

Total number of observations = 4

Total number of records with missing values = 0

Maximum number of iterations = 500

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values

background =	0
intercept =	-3.54742
slope =	1
~~~~~~~~~~

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.46
intercept	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.225662	*	*	*
intercept	-58.2858	*	*	*
slope	18	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-1952.54	4			
Fitted model	-1952.66	2	0.247206	2	0.8837
Reduced model	-1956.17	1	7.26439	3	0.06393

AIC: 3909.32

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
4.2000	0.2257	202.193	202.000	896	-0.015
7.5000	0.2257	201.516	206.000	893	0.359
12.4000	0.2257	201.292	197.000	892	-0.344
21.8000	0.2696	241.000	241.000	894	0.000

Chi^2 = 0.25 d.f. = 2 P-value = 0.8837

Benchmark Dose Computation

Specified effect = 0.01

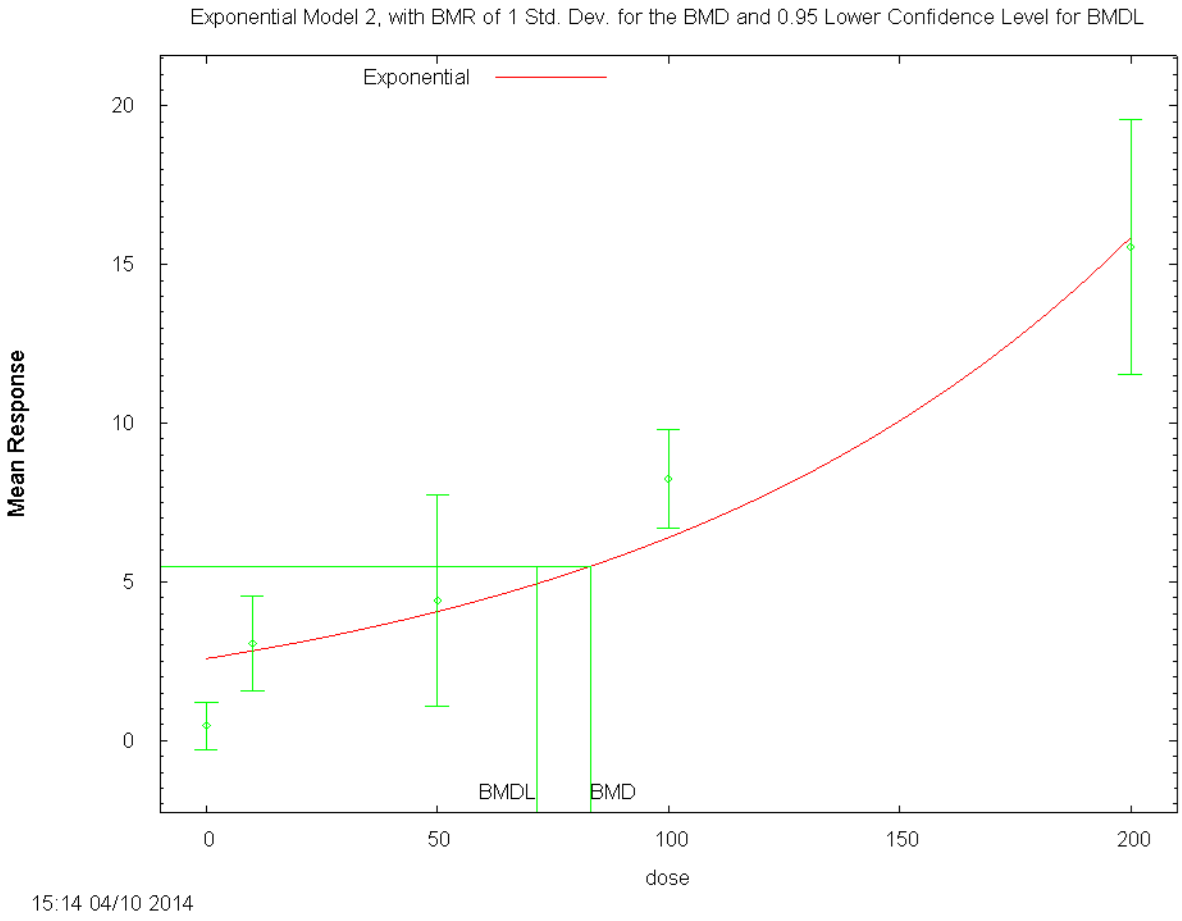
Risk Type = Extra risk

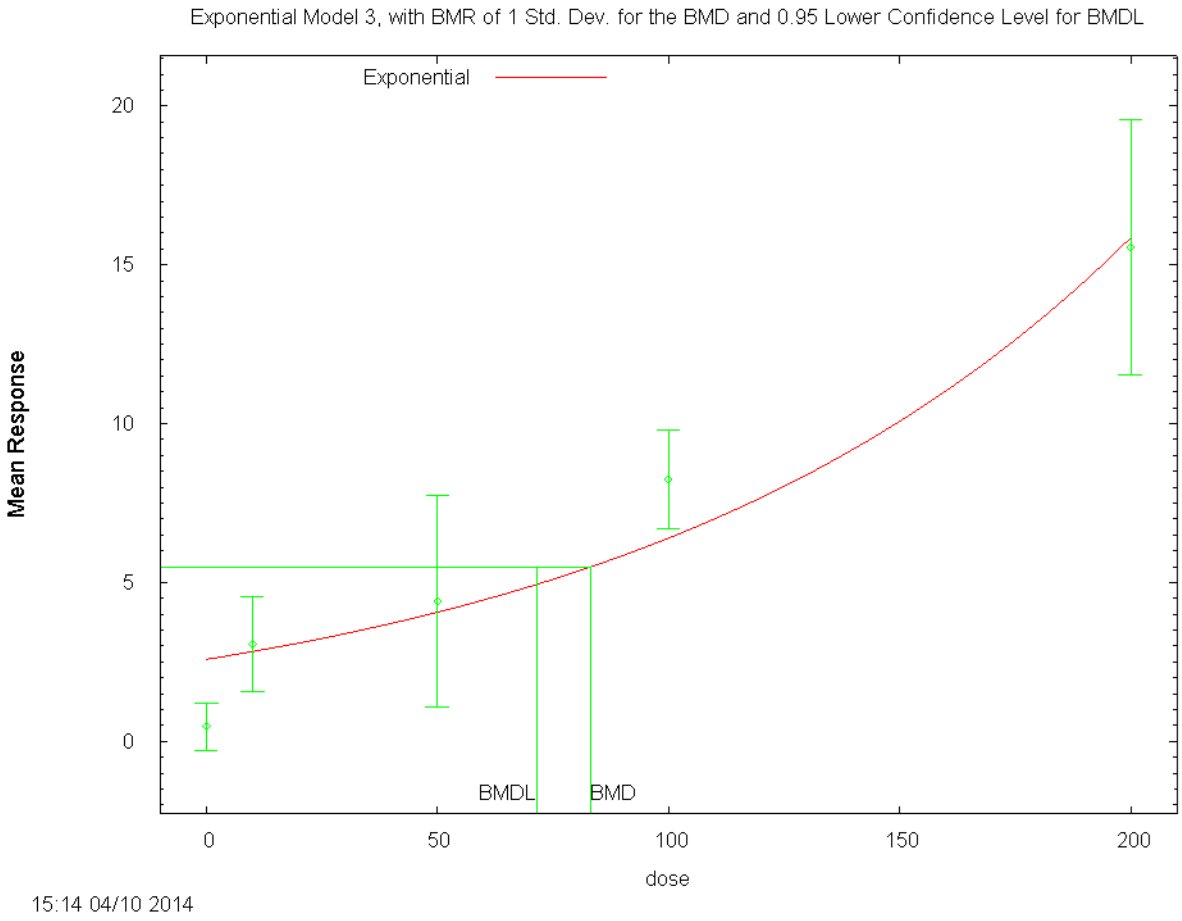
Confidence level = 0.95

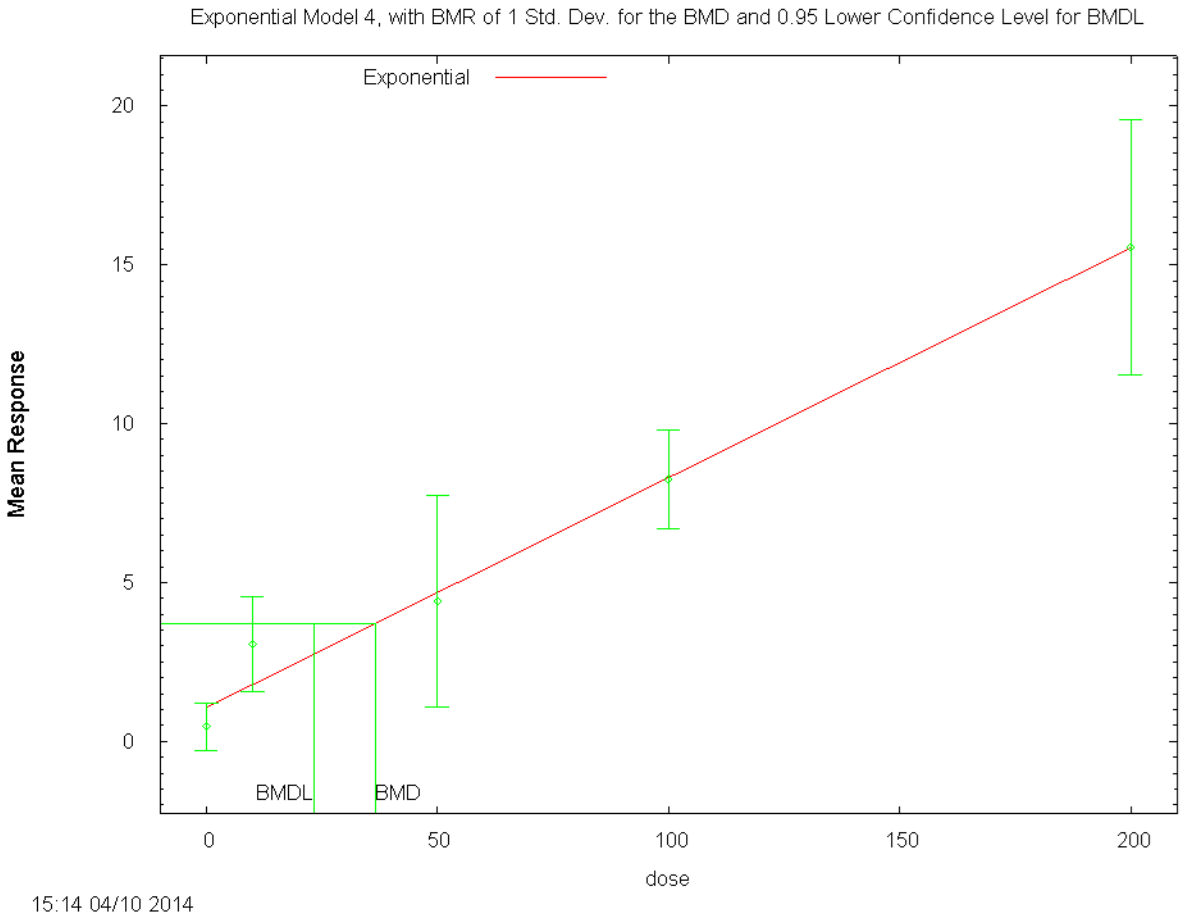
BMD = 19.7433

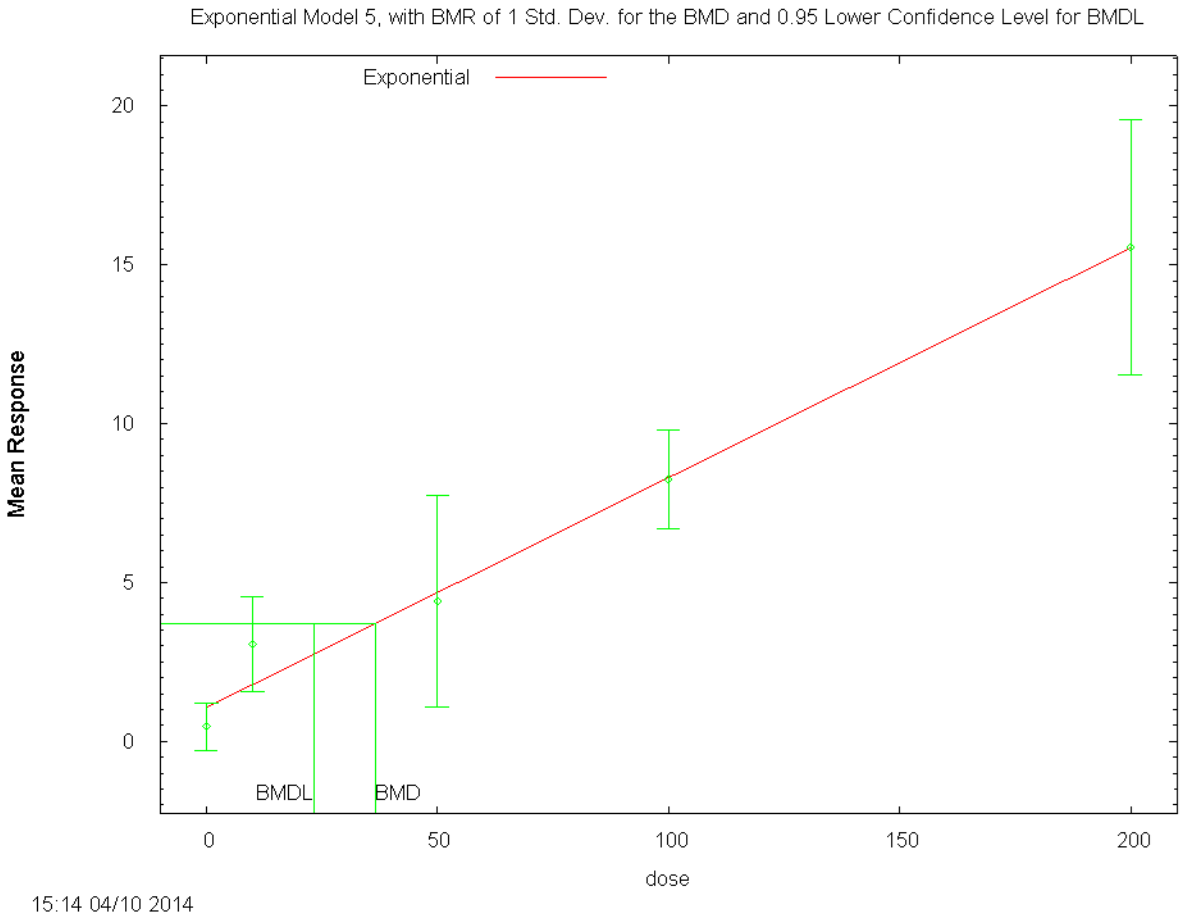
BMDL = 2.24123

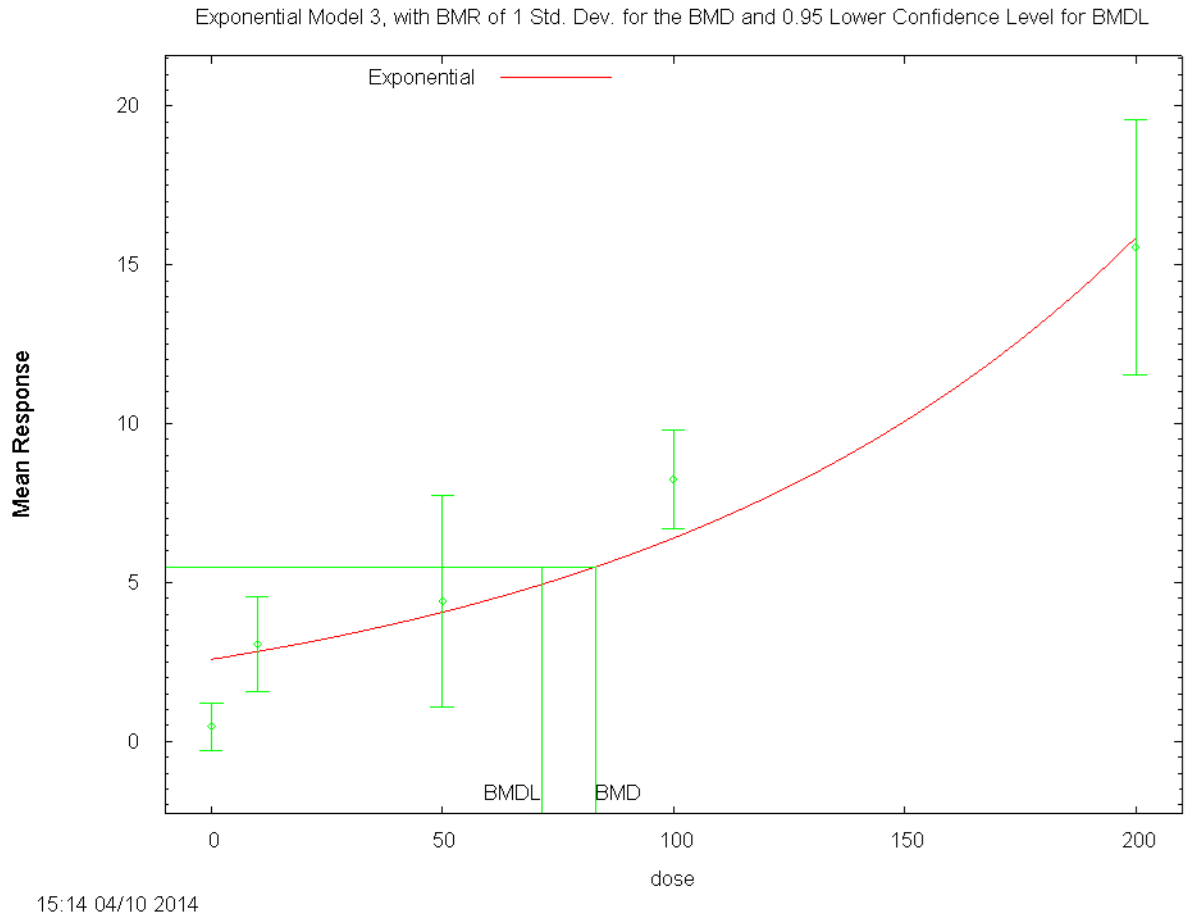
USEPA BMD software output for mouse data for Exponential models (Best-fit)




Exponential Model 3, with BMR of 1 Std. Dev. for the BMD and 0.95 Lower Confidence Level for BMDL
Exponential
Mean Response
20
15
10
5
0
BMDL
BMD
0
50
100
150
200
dose
15:14 04/10 2014


Exponential Model 4, with BMR of 1 Std. Dev. for the BMD and 0.95 Lower Confidence Level for BMDL
Exponential
Mean Response
20
15
10
5
0
BMDL
BMD
0
50
100
150
200
dose
15:14 04/10 2014


Exponential Model 5, with BMR of 1 Std. Dev. for the BMD and 0.95 Lower Confidence Level for BMDL
Exponential
Mean Response
20
15
10
5
0
BMDL
BMD
0
50
100
150
200
dose
15:14 04/10 2014




```
================================================================================
        Exponential Model. (Version: 1.9;  Date: 01/29/2013)
        Input Data File: C:/BMDS/BMDS240/Data/exp_Dax_Setting.(d)
        Gnuplot Plotting File:
                                          Thu Apr 10 15:14:54 2014
 ================================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
```

Model 2: Y[dose] = a * exp{sign * b * dose}

Model 3: Y[dose] = a * exp{sign * (b * dose)^d}

Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]

Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;

sign = +1 for increasing trend in data;

sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.

Model 3 is nested within Model 5.

Model 4 is nested within Model 5.

Dependent variable = Mean

Independent variable = Dose

Data are assumed to be distributed: normally

Variance Model: exp(lnalpha +rho *ln(Y[dose]))

rho is set to 0.

A constant variance model is fit.

Total number of dose groups = 5

Total number of records with missing values = 0

Maximum number of iterations = 500

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	1.89729	1.89729	1.89729	1.89729
rho(S)	0	0	0	0
a	1.18617	1.18617	0.439375	0.439375
b	0.0143973	0.0143973	0.001086	0.001086
c	--	--	177.07	177.07
d	--	1	--	1

(S) = Specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	2.13807	2.13807	1.94856	1.94856
rho	0	0	0	0
a	2.58405	2.58405	1.06638	1.06642
b	0.00907074	0.00907074	2.70896e-006	2.37356e-006
c	--	--	25055.7	28594.1
d	--	1	--	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	8	0.4625	0.9054
10	5	3.05	1.21

50	5	4.42	2.69
100	6	8.25	1.475
200	8	15.56	4.81

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	2.584	2.913	-2.06
	10	2.829	2.913	0.1694
	50	4.067	2.913	0.271
	100	6.401	2.913	1.555
	200	15.86	2.913	-0.2868
3	0	2.584	2.913	-2.06
	10	2.829	2.913	0.1694
	50	4.067	2.913	0.271
	100	6.401	2.913	1.555
	200	15.86	2.913	-0.2868
4	0	1.066	2.649	-0.6447
	10	1.79	2.649	1.063
	50	4.685	2.649	-0.2237
	100	8.303	2.649	-0.04917
	200	15.54	2.649	0.02348
5	0	1.066	2.649	-0.6448
	10	1.79	2.649	1.063
	50	4.685	2.649	-0.2236
	100	8.303	2.649	-0.04909
	200	15.54	2.649	0.02343

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)

Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)

Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)

Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-46.35666	6	104.7133
A2	-33.27237	10	86.54474
A3	-46.35666	6	104.7133
R	-74.76534	2	153.5307
2	-50.20914	3	106.4183
3	-50.20914	3	106.4183
4	-47.17703	4	102.3541
5	-47.17703	4	102.3541

Additive constant for all log-likelihoods = -29.41. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)

Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)

Test 7b: Is Model 5 better than Model 3? (5 vs. 3)

Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	82.99	8	< 0.0001
Test 2	26.17	4	< 0.0001
Test 3	26.17	4	< 0.0001
Test 4	7.705	3	0.05252
Test 5a	7.705	3	0.05252
Test 5b	-9.948e-014	0	N/A
Test 6a	1.641	2	0.4403
Test 6b	6.064	1	0.01379

Test 7a	1.641	2	0.4403
Test 7b	6.064	1	0.01379
Test 7c	3.997e-006	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. Consider running a non-homogeneous variance model.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0.
The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

Degrees of freedom for Test 7c are less than or equal to 0. The Chi-Square test for fit is not valid.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	83.2099	71.4066
3	83.2099	71.4066
4	36.6051	23.392
5	36.6061	23.392

APPENDIX E. LIST OF ABBREVIATIONS

AIC	Akaike's information criterion
ApoE–/–	Apolipoprotein E-knockout
ATSDR	Agency for Toxic Substances and Disease Registry
BFDEA	Blackfoot disease endemic area
BMD	Benchmark Dose
BMDL	Benchmark Dose lower bound
BMR	Benchmark Response
CAD	Coronary artery disease
CHD	Coronary heart disease
CVD	Cardiovascular disease
DALY	Disability adjusted life years
EFSA	European Food Safety Authority
FERG	Foodborne Disease burden Epidemiology Reference Group
GBD	Global burden of disease
GEMS	Global Environment Monitoring System
IARC	International Agency for Research on Cancer
iAs	Inorganic arsenic

IRIS	Integrated risk information system
JECFA	Joint Expert Committee on Food Additives
LOAEL	Lowest observed adverse effect level
MI	Myocardial infarction
MLE	Maximum likelihood estimates
MOA	Mode of action
NOAEL	No observed adverse effect level
NMSC	Non-melanoma skin cancer
NRC	National Research Council
OR	Odds ratio
POD	Point of departure
PTWI	Provisional tolerable weekly intake
PVD	Peripheral vascular disease
PYR	Person years at risk
RfD	Reference dose
RR	Risk ratio
SAM	S - Adenosyl Methionine
SD	Standard deviation
SE	Standard Errors
SMR	Standardized mortality rate
UF	Uncertainty factor
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

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