ASSESSMENT OF PUBLIC HEALTH RISK FROM ENVIRONMENTAL TOXICANT USING BIOMARKERS AND BIOKINETICS MODELING

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Furxhi Irini

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Assessment of public health risk from environmental toxicant using biomarkers and biokinetics modeling

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***Dimosthenis A. Sarigiannis**, M.Sc., PhD (University of California, Berkeley, USA) Associate Professor specializing on environment health issues at the Department of Chemical Engineering of the Aristotle University of Thessaloniki, Visiting Professor at the Master's Program on Toxicology and Environmental Risk at the Medical School of the University of Pavia and senior scientist at the Chemical Assessment and Testing unit of the Institute for Health and Consumer Protection at the European Commission's Joint Research Centre (currently on leave).*

***Spyros Karakitsios** B.Sc., M.Sc., PhD, Head of Exposure Biology Unit. environmental health scientist at the Department of Chemical Engineering of the Aristotle University of Thessaloniki*

***Christina Tsitsimpikou**, M.Sc., PhD, Toxicological and Clinical Analyst and Administrator, Senior Officer, RAC member for health issues European Chemical Agency – REACH/CLP enforcement, General Chemical State Laboratory of Greece*

SUMMARY

Arsenic is a metalloid which occurs in various forms (organic and inorganic) and concentrations in environment from natural occurrence, but also from anthropogenic activity. From a biological and toxicological perspective, there are three major groups of arsenic compounds: inorganic (arsenic combined with elements such as: oxygen, sulfur, chlorine), organic (combined with carbon and hydrogen) and arsine gas (the most toxic). Human exposure to arsenic can occur over a lifetime, from water and food consumption, as well as from exposure to soil, dust, air, and breast milk (EFSA, 2009; Rebelo and Caldas, 2016). Arsenic ranks first on the Agency for Toxic Substances and Disease Registry - ATSDR (ATSDR, 2015) meaning it manifest great toxicological concern to humans. From the various forms of arsenic, the most harmful to human health are the inorganic forms. Inorganic arsenic is associated with numerous adverse effects in humans, both cancerous and non-cancerous.

Arsenic contamination in drinking water is a major environmental issue. Many populations across the world, particularly, India and Bangladesh, face a public health crisis from their use of groundwater, which is contaminated with high levels of Arsenic, as their source of drinking water (Alam et al., 2002; Khan et al., 2003). Recent studies made in Greece have identified increased contamination in some areas. An appropriate understanding of the chemical properties of arsenic and its behavior to the environment and human body, are critical in predicting and estimating human health related risks. Using a Biologically Based Dose Response (BBDR) model we can estimate the potential individual risk and consequently population health risk from the internal dose or else, biologically effective dose, obtained from a Physiologically Based Pharmacokinetic (PBPK) model. PBPK models can describe the mechanisms of absorption, distribution, metabolism and elimination (ADME) of chemicals in the body allowing us to obtain the internal concentration of arsenic to the target organs.

In this case, a PBPK model which takes into consideration distribution within human body of both inorganic arsenic (Arsenite and Arsenate) and the two main metabolites (Dimethylarsinate and Monomethylarsonate) was used. Coupling a PBPK model with a Biological Based Dose Response Model, allowed the quantitative estimation of health risk in individual and population level, due to aggregate arsenic exposure. In addition, the use of the PBPK model allowed the utilization of Arsenic biomonitoring data for reconstructing exposure and then estimate the associated As related risks in global scale. The challenge of this study was the connection of dose-response modelling approaches no longer to the external concentration of arsenic, but to the internal exposure at the different target organs, accounting for gender susceptibility differences as well. Based on the water contamination levels, the estimated risks ranged from 10^{-7} to 10^{-4}, depending on the contamination levels.

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ABBREVIATIONS

As(i)	Inorganic arsenic
As^{III}	Trivalent inorganic arsenic, Arsenite, Arsenious acid
As^{V}	Pentavalent inorganic arsenic, Arsenate, Arsenic acid
MMA	Methylarsonic acid, monomethylarsonic acid
MMA^{III}	Monomethylarsonous acid
MMA^{V}	Monomethylarsenic acid
DMA	Dimethylarsinic acid, Cacodylic acid
DMA^{III}	Dimethylarsinous acid
DMA^{V}	Dimethylarsinic acid
TMAO	Trimethylarsine oxide
AS3MT	Arsenic +3 oxidation state methyltransferase
GSTO1	Glutathione -S-transferase omega
SAM	S-adenosyl methionine
GSH	Glutathione
GST	Glutathione-S-transferase
GI	Gastrointestinal
ATSDR	Agency for Toxic Substances and Disease Registry
PBPK	Physiologically based pharmacokinetic
ADME	Absorption, Distribution, Metabolism and Excretion
CAS	Chemical Abstract Service
RBCs	Red Blood Cells
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
IARC	International Agency for Research on Cancer
EPA	Environmental Protection Agency, United States of America
WHO	World Health Organization
MCL	Maximum Contaminant Level
NOAEL	No-observed-adverse-effects-levels
LOAEL	Lowest-observed-adverse-effect-levels
BMDL	Benchmark Dose Lower Confidence Limit

INTRODUCTION

ARSENIC

GENERAL INFORMATION OF ARSENIC

Arsenic is a metalloid existing in Group 15, number 33 of the periodic table, which means it has properties in between those of metals and nonmetals. Inorganic arsenic is a naturally occurring metalloid found in water, air, soil, many kind of rocks such as volcanic rock, minerals, ores, organic matter and food (Rebelo and Caldas, 2016). The origin of Arsenic begins from red giant stars and supernovas, rather than from the Big Bang (Henke, 2009). Arsenic exists in -3, 0, +3, and +5 valence oxidation states (Mohan and Pittman, 2007) and in a variety of chemical forms (organic and inorganic) resulting its complexity of chemistry in the environment. Inorganic arsenic is comprised of arsenite (trivalent form) and arsenate (pentavalent form) (Georgopoulos et al., 2007). The most common trivalent inorganic arsenic compounds are: arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$), and arsenic trichloride ($AsCl_3$). It is generally accepted that the inorganic species, As^{III} and As^{V}, are the predominant species in most environments (Andrianisa et al., 2008). The pentavalent arsenic pentoxide (As_2O_5) has high solubility in water so we assume that the chief (EPA, 1984) chemical form of inorganic arsenic in public water supplies would be the pentavalent inorganic form. Inorganic arsenic is generally found in drinking water as either arsenate As^{V} or arsenite As^{III}. As^{V} is found primarily in oxygenated waters in his stable form, whereas As^{III} is detected more frequently in reducing or low oxygen environment (Postma et al., 2007). Although As^{V} tends to be less toxic compared to As^{III}, it is thermodynamically more stable due to it predominates under normal conditions and becomes the cause of major contaminant in groundwater (Chutia et al., 2009). Most arsenic compounds are colorless, tasteless, odorless powders that do not evaporate. Thus, it i usually difficult to tell if arsenic is present in the food, water, or air (ATSDR, 2007).

BIOTRANFORMATION

Arsenic cannot be destroyed in the environment but it can only change forms by reacting with several elements or molecules present in the environment, or even by the action of bacteria that live in soil or sediment. Arsenic can get into lakes, rivers, or groundwater by dissolving in rain or snow or thought the discharge of industrial wastes. Arsenic species have an affinity for clay mineral surfaces and organic matter and this can affect their environmental behavior. Bioaccumulation of arsenic in the aquatic environment is dependent on environmental conditions, trophic status within the food chain and route of uptake (Williams et al., 2006). Three major modes of arsenic biotransformation have been found to occur in

the environment: (1) redox transformation between arsenite and arsenate, (2) reduction and methylation of arsenic, and (3) biosynthesis of organoarsenic compounds. From these processes, a complex biogeochemical cycling of compounds is formed (WHO. et al., 2001; Williams et al., 2006).

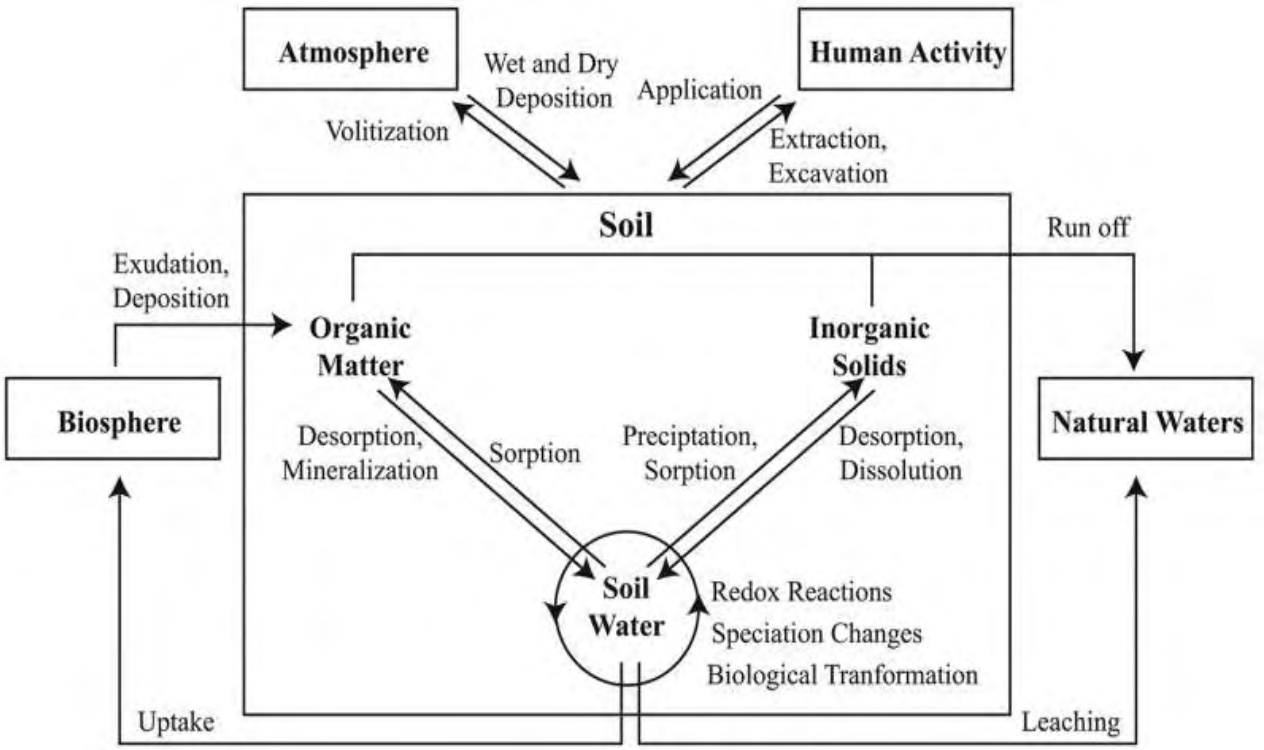

Figure 1. Core concepts of Arsenic biogeochemical cycling.

As shown in the Fig.1 Arsenic is released into the atmosphere (primarily as As_2O_3) where it exists mainly absorbed on particulate matter. These particles are dispersed by the wind and are returned to the earth (wet or dry deposition) (O.W. and J.M., 2012). Arsines released from microbial sources in soils or sediments undergo oxidation in the air (Tamaki and Frankenberger, 1992), reconverting the arsenic to non-volatile forms (Parris and Brinckman, 1976), which settle back to the ground. Natural low-temperature biomethylation and reduction to arsines also releases arsenic into the atmosphere. When found in the soil arsenic can undergo biological transformation ending up in groundwater. Right geochemical conditions will control whether arsenic will leach in water i.e. water with high pH or relatively little dissolved oxygen. About 60% of anthropogenic arsenic emissions to the global atmosphere originate from flue gases emitted by copper ore smelter and coal-combustion facilities (Henke, 2009). Arsenic is emitted into the atmosphere by high-temperature processes such as coal-fired power generation plants, burning vegetation and volcanism (human and natural activity). World arsenic production in the year 2008 was estimated to be 53,500 tonnes (As_2O_3), whereof less than 1,500

tonnes was estimated to be produced within the EU (Chain, 2009; USGS, 2014).Global natural emissions have been estimated to be 7900 tonnes per year, while anthropogenic emissions are about three times higher. Improvements of industrial processes have led to substantial decreases of the emissions of arsenic from the metal industry.

EXPOSURE

Exposure to arsenic for the general population may occur through inhalation (contaminated air due to industrial emissions or flue gas from coal-combustion power plants and ore smelters, cigarette smoking), dermal absorption (CCA-treated wooden decks and playground structures (Hemond and Solo-Gabriele, 2004), ingestion of food, water and soil in the environment, while occupational exposure to arsenic may occur from production of wood preservatives, herbicides, and insecticides (Henke, 2009). Human exposure to arsenic occurs primarily through the consumption of water and seafood, particularly shellfish (EFSA, 2009). The most common long term exposure to inorganic arsenic is contaminated water. From the forms of Arsenic in drinking water, and its resultant metabolism, an individual may be exposed internally to at least six different arsenicals (Cohen et al., 2007). As said above, arsenic occurs in the environment in different forms but inorganic arsenic is the most toxic one. Inorganic arsenic is mostly found in meats, poultry, dairy products, mushrooms, tea and cereals (Velez et al., 1996). The major food contributors to inorganic arsenic are vegetables (24%), fruits (18%), rice (17%), beer and wine (12%). Approximately 10% of the total arsenic exposure from foods is the inorganic toxic form. Like all the molecules found in the universe, once arsenic is found in our body, a biological process begins which aims its elimination from our system. These process is called “ADME”, a pharmacokinetic abbreviation for absorption, distribution, metabolism and excretion.

DESCRIPTION OF ARSENIC KINETICS

ABSORPTION

Absorption is defined as the process by which a drug proceeds from the site of administration to the site of measurement (usually blood, plasma, or serum). When Arsenic enters the body barriers is readily transported to the cell (Schuhmacher-Wolz et al., 2009) where it binds to hemoglobin, plasma proteins and leukocytes. Then is redistributed to the liver, kidney, lung, spleen and intestines (WHO, 2007). Arsenic can cross cell membranes by passive diffusion or carrier protein mediated transport (Mann et al., 1996a) and bond to intracellular components, favoring its accumulation (Georis et al., 1990). Absorption from food is assumed to occur primarily in the small intestine (Henke, 2009) where is nearly completely absorbed (80%) after ingestion (Duker et al., 2005). The absorption of arsenic

from the GI tract to the liver is described by using first-order kinetics. As concern the skin, dermal absorption of inorganic arsenic residue on the surface of objects is low (Wester et al., 1993) dermal exposure is assumed to be insignificant in this study, but inorganic arsenic may accumulate in skin, bone, liver and kidney.

DISTRIBUTION

After being absorbed, arsenic is widely distributed to almost all organs. In the bloodstream, arsenic is distributed between the plasma and the erythrocytes. Only metabolites present in the plasma are considered available for distribution to the tissues, because arsenic bounds to RBCs, therefore considered unavailable for exchange with tissues (Mann et al., 1996a). The form of the Physiologically Based PharmacoKinetic models (*This will be discussed later in this essay*) depends predominantly on the rate of the tissue/blood distribution of the compounds (Baláž and Lukáčová, 1999). When all the incoming compound is available for distribution in the tissues, this behavior is referred as *perfusion-limited uptake* (Andersen, 1991).

METABOLISM

It is likely that metabolism of arsenic, like other toxic metals, is associated with the conversion of the most potentially toxic forms of this element to the less toxic form, followed by accumulation in or excretion from the cell. Two metabolic pathways for As(i) have been described, an enzymic arsenic reduction/methylation pathway (Buchet and Lauwerys, 1985) and an alternative pathway involving nonenzymatic formation of arsenic-glutathione complexes. Fig.2 next page depicts the Arsenic metabolic pathways used later in the PBPK model formulation.

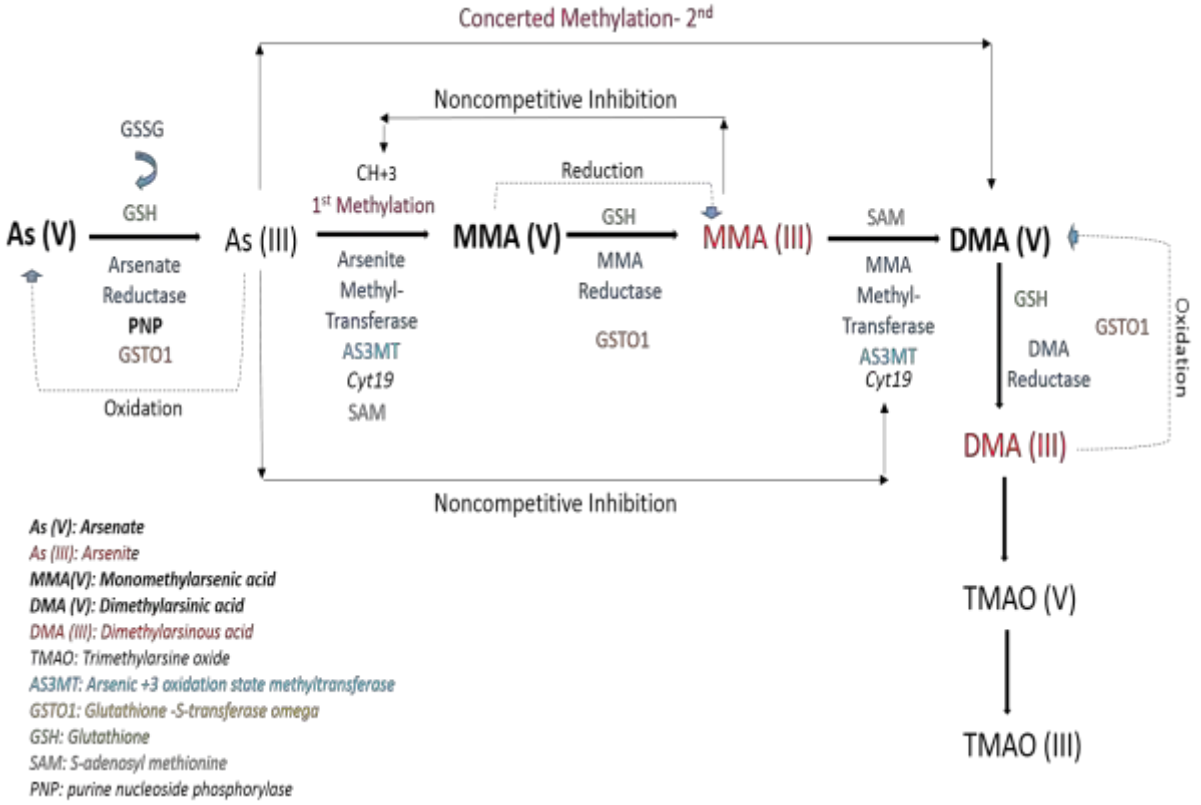

Figure 2. Arsenic metabolism pathways.

As seen in Figure 2, Reduction of AsV produces AsIII which is a substrate for AS3MT to methylate, to form MMAV. MMAV is reduced to MMAIII which is methylated by AS3MT (or using SAM as a methyl donor) to form DMAV. DMAV is further reduced to form DMAIII. The modelled metabolic pathways included in addition rates of oxidation of trivalent arsenicals to their respective pentavalent forms. A third metabolic pathway has recently been described where involves initial binding of inorganic arsenic to sulfhydryl groups of cysteinyl moieties on proteins, followed by reductive methylation catalyzed by AsIII, AS3MT and using the methyl group donor SAM to form MMAV and DMAV (ATSDR, 2007) Quantitative description of arsenic metabolic pathways is further complicated by the inhibitory influence of metabolites on methylation (Easterling et al., 2002; Kenyon et al., 2001; Styblo et al., 1996).

Reduction of As^{V}, MMA, and DMA^{V} takes place very rapidly and can occur by either enzymatic or non-enzymatic mechanisms (El-Masri and Kenyon, 2008a; Zakharyan et al., 2005). Mitochondria can work as reactors, where they take up As^{V}, rapidly reduce it, and export the formed As^{III} (Németi and Gregus, 2002). Methylation of inorganic arsenic facilitates the excretion of inorganic arsenic from the body, as the end-products are readily excreted in urine, for this reason, the methylation of arsenic was viewed as a detoxification pathway (Buchet and Lauwerys, 1985). However, the methylation of inorganic arsenic may

be a toxificaton-activation process, due to the great biological activity of trivalent methylated arsenic metabolites with proteins and even DNA (Kitchin, 2001).

A single enzyme has been identified, AS3MT, that catalyzes both the oxidative methylation of trivalent arsenicals and the reduction of pentavalent arsenicals (Waters et al., 2004a; Waters et al., 2004b) but also others enzymes support those processes, such as GSTO1 (Chowdhury et al., 2006; Zakharyan and Aposhian, 1999), which is widely distributed in human tissues. Recently a new enzyme AdoMet dependent methyltransferase (Thomas et al., 2004) has been reported.

Two genes are responsible for arsenic metabolism: human nucleoside phosphorylase (hNP) and human glutathione S-transferase omega 1-1 (hGSTO 1-1), (Yu et al., 2003). Polymorphisms in those genes have been discovered. Studies in humans suggest the existence of a wide difference in the activity of methyl-transferases, and the existence of polymorphisms. Genetic polymorphism that have been examined include AS3MT, cystathione-β-synthase, Glutathione-S-transferase π1, ω1, methylenetetrahydrofolate reductase, and N-6 adenine- specific DNA methyltransferase 1 (ATSDR, 2007). Individuals with polymorphisms associated with a higher MMA: DMA ratio in urine may be more susceptible to arsenic-induced toxicity.

Children seem to have their own way dealing with arsenic. The first metabolic pathway is more active in adults than children, but the second methylation step is more active in children than adults (Chowdhury et al., 2003). Due to this reason, fetuses and babies may be protected by increased methylation of arsenic during pregnancy and breastfeeding (Gurbay et al., 2012). Fängström et al. (2008) found that arsenic in blood plasma does not pass easily through the mammary glands and arsenic in breast milk correlated negatively with DMA%. Thus, indicating that breast-feeding protects the infant from exposure to arsenic (Fängström et al., 2008). The same conclusion came also from Carignan et al. (Carignan et al., 2015).

EXCRETION

Arsenic is excreted in the urine primarily through the kidneys. Humans excrete a cocktail of inorganic, monomethylated and dimethylated forms of arsenic. The pentavalent metabolites MMAV and DMAV are less toxic than arsenite or arsenate (ATSDR, 2011). Approximately 50% of excreted arsenic in human urine is dimethylated and 25% is monomethylated, with the remainder being inorganic. Other less important routes of elimination of inorganic arsenic include feces, incorporation into hair and nails, skin desquamation, and sweat. The whole-body biological half-life of ingested arsenic is about 10 hours, and 50-80% is excreted over 3 days (Casarett and Klaassen, 2008).

MODE OF ACTION

The toxicity of arsenic, including cancer, is most likely due to multiple mechanisms. The mechanisms responsible for the adverse effects associated with arsenic, probably occur through multiple independent and interdependent mechanisms (Duker et al., 2005; NRC, 2001). Two general types of mechanisms appear to be involved in arsenic-induced toxicity: (1) formation of reactive oxygen species (ROS). Arsenic can disrupt the oxidative phosphorylation, leading to free radical formation. Pentavalent arsenic may be transformed to a substitute for inorganic phosphate in glycolysis, leading to uncoupling of oxidative phosphorylation and loss of ATP formation (TOXNET, 2016). Arsenic-induced ROS generation has been associated with numerous effects on cellular targets (Hubaux et al., 2013), which can directly damage cellular components or lead to a cascade of effects in response to oxidative stress (alterations in intracellular oxidation/reduction reaction, decreased glutathione levels, lipid peroxidation, damage to proteins, disruption of mitochondrial membrane, genomic instability through damage to DNA). The current consensus in studies with cultured cells, experimental animals, and humans is the fact that arsenic causes oxidative stress through the generation of reactive oxygen species (Fujino et al., 2005; Kumagai and Sumi, 2007). (2) interaction of arsenic metabolites with cellular macromolecules. Arsenic can interfere with essential enzymatic functions and transcriptional events in the cells. Inorganic arsenic exerts epigenetic effects (Bodwell et al., 2006; Reichard et al., 2007). Trivalent species are more potent cytotoxicants, genotoxicants and inhibitors of enzymes compared to pentavalent arsenicals (El-Masri and Kenyon, 2008a). One of possible mechanisms for higher toxicity is the higher affinity for thiol compounds (Shiobara et al., 2001) and generation of reactive oxygen species (Nesnow et al., 2002). Exposure to inorganic arsenic has been shown to modify the expression of a variety of genes related to cell growth and defense, including the tumor suppressor gene p53, as well as to alter the binding of nuclear transcription factors (TOXNET, 2016).

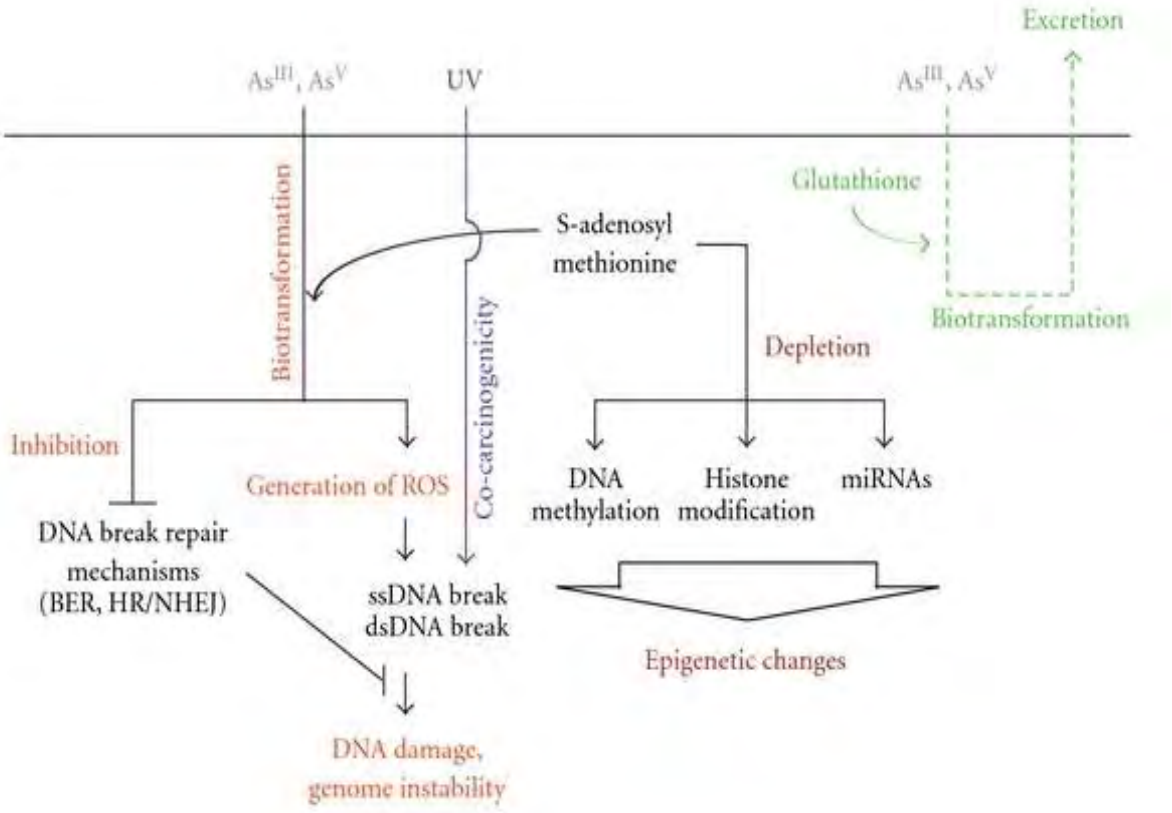

Figure 3. Carcinogenic mechanisms of arsenic transformation.

Fig.3 explains how ingested arsenic undergoes biotransformation process and how those can result to carcinogenic activity. (1) Biotransformation could lead to arsenic excretion, when conjugated with glutathione. (2) Biotransformation generates reactive oxygen species (ROS), that induce single-strand (ssDNA) and double-strand (dsDNA) breaks by inducing oxidative damage. The process can also inhibit DNA break repair mechanisms (Martinez et al., 2011). Additionally, ROS can act as co-carcinogens. Furthermore, the requirement of S-adenosyl methionine (SAM) for arsenic biotransformation can lead to depletion of SAM, which is the substrate for DNA methylation. Recently, a study showed that exposure to arsenic triggers a shift in microRNA expression and revealed an induction of cell cycle progression and failure of apoptosis supporting the idea of inorganic arsenic carcinogenic activity (Sturchio et al., 2014).

Unlike many carcinogens, arsenic is not a mutagen in bacteria and acts weakly in mammalian cells, but can induce chromosomal abnormalities, aneuploidy, and micronuclei formation. In vitro studies showed that As^{III} exposure to humans from drinking water can lead to the formation of micronuclei (Johnson, 2007). Arsenic can also act as a co-mutagen and/or co-carcinogen (Casarett and Klaassen, 2008). Although a large amount of research is available on arsenic's mode of action, the exact nature of carcinogenic action is not yet clear

(NRC, 2001). The proposed Mode of Action include alteration in DNA repair, change in DNA methylation, suppression of cell cycle check point protein (p53), altered expression of growth factor and oxidative stress.

ADVERSE EFFECTS

CARCINOGENICITY

Inorganic arsenic has been classified by the IARC (IARC, 1973) in Group 1 as carcinogenic to humans on the basis of increased incidence of cancers at several sites where people were exposed. IARC (2004) has classified arsenic as a known human carcinogen, associated with tumors of the skin, lung, and urinary bladder, and possibly kidney, liver, and prostate. A ranging risk of 10^{-4} to 10^{-7} was developed by EPA (ATSDR, 2007). An established association between human arsenic exposure and human cancer has been known for many years (Chen et al., 1992; Wu et al., 1989). A clear dose-response relation between Arsenic and drinking water for cancer in kidney, lung and bladder has been reported in Argentina (Hopenhayn-Rich et al., 1998) and a high lung cancer mortality in Japan (Tsuda et al., 1995). Arsenic is contributing to cancer (Bernstam and Nriagu, 2000; Clewell et al., 1999) of the skin (Yu et al., 2000), lungs (Ferreccio et al., 2000; Lubin et al., 2000), kidney, liver and bladder (Bates et al., 1992; Chen and Wang, 1990; Smith et al., 1992). Trivalent methylated arsenicals are responsible for the toxicity and carcinogenicity of environmental arsenic (Hirano et al., 2004; Nesnow et al., 2002). MMA^{III} and DMA^{III} have been suggested as potential contributors to arsenic-induced carcinogenicity (Bernstam and Nriagu, 2000; Kitchin, 2001). DMA^{V} on the other hand, is a urinary bladder carcinogen and tumor promoter in rats (Cohen et al., 2006). The most common pathway of exposure to inorganic arsenic for the general population is via the drinking water. Early effects of exposure to arsenic in drinking water included pigmentation changes and hyperkeratosis (Alam et al., 2002; Mazumder et al., 1998; Smith et al., 2002b). These skin lesions may develop into more serious and disabling forms, including cancer (Haque et al., 2003). In the Table below, several endpoints concerning exposure to inorganic arsenic and cancer are summarized.

Table 1. Toxicological cancer endpoints for inorganic arsenic using evidence from human studies

Chronic exposure - Inhalation			
Exposure	LOAEL	Form	Ref.
1->30y	0.213M (serious) CEL: lung cancer	AsIII	Enterline et al. 1987a
19.5y	0.064M (serious) CEL: lung cancer 0.064 mg/kg/day for liver and lung cancer corresponds to 4,48 mg/day for a weight of 70 kg	AsIII	(Enterline et al., 1987)
3m->30y	0.05M (serious) CEL: lung cancer (3.5mg/day)	AsIII	(Jarup et al., 1989)
1->30y	0.38M (serious) CEL: lung cancer	AsIII	(Lee-Feldstein, 1986)
>25y	0.29M (serious) CEL: lung cancer	AsIII	(Lubin et al., 2000)
14.8y	0.3M (serious) CEL: lung cancer	AsIII	(Welch et al., 1982)
Intermediate Oral Exposure			
0.5-14y	0.05 (serious) hyperpigmentation with keratosis, possibly pre-cancerous		(Huang et al., 1985)
4mo	0.06F (serious)persistent extensive hyperkeratosis of palms and soles		(Wagner et al., 1979)
Systemic Oral Exposure			
>8y	0.0012 (less serious) increased risk of premalignant skin lesions		(Ahsan et al., 2006)
4y	0.1 F (serious) de-pigmentation with hyperkeratosis, pre-cancerous	As(III)	(Bickley and Papa, 1989)
NS	0.009 (serious) hyperpigmentation with keratosis, pre-cancerous		(Guha Mazumder et al., 1988)
Cancer			
NS	0.0011 CEL: lung cancers (0.077 mg/day)		(Ferreccio et al., 2000)
NS	0.018 CEL: lung cancer mortality 1.26 mg/day		(Guo, 2004)
NS	0.018 CEL: bladder cancer		Guo and Tseng 2000
>1y	0.0075 CEL: basal or squamous skin carcinoma (0.525 for 70kg)		(Haupert et al., 1996)
5y	0.033 CEL: lung, urinary tract cancer 2.31 mg/day for a weight of 70 kg	As(III)	(Tsuda et al., 1995)
22-34y	0.014 CEL: basal cell and squamous cell carcinomas of the skin, hemangio endothelioma of the liver 0.014 mg/kg/day for fatal liver tumor and 22 year of exposure, corresponds to 0.98 mg/day for a weight of 70 kg		(Zaldivar et al., 1981)

Despite all the information about carcinogenesis to human beings, development of a reliable animal model system for arsenic-induced carcinogenicity has been difficult (Ng et al., 1999), indicating marked variation in sensitivity towards arsenic toxicity between species (Vahter, 1999). The difficulties in this area could be due to species-specific differences in detoxification, metabolism, or uptake and accumulation in target tissues. There are major qualitative and quantitative interspecies differences, for example in methylation (Hsueh et al., 1998; Mann et al., 1996b). Another example is the Trimethylarsine oxide (TMAO), the final metabolite of inorganic arsenic in some animal species, but has never been found in human urine (Yoshida et al., 1997). Some animal species even lack arsenic methylation capacity, perhaps as an adaptation mechanism (Casarett and Klaassen, 2008). Only in the last decade has the metal been demonstrated to cause cancer in animals under specific exposure scenarios.

OTHER HEATH IMPACTS

Epidemiological studies have indicated that ingested inorganic arsenic is associated with chronic diseases such as dermal, cardiovascular, neurological effects and mellitus diabetes (Chiou et al., 2001; EPA, 2005; Lamm et al., 2006; O'Bryant et al., 2011; Smith et al., 2002a). Inorganic arsenic can cause reproductive toxicity, including increases in fetus mortality, underweight newborns, spontaneous abortions, eclampsia, and birth defects (Rebelo and Caldas, 2016). Exposure during childhood or in the uterus may have adverse reproductive outcomes for mothers inducing changes in cognitive development of children. Developmental and neurodevelopmental effects have been observed in infants and children following prenatal and early life exposure to arsenic in drinking water ((ATSDR). 2007). Arsenic may cause Raynaud's phenomenon (Kumagai and Sumi, 2007). Studies in Chile indicate that ingestion of 0.6-0.8 ppm As in drinking water (corresponding to doses of 0.02-0.06 mg As/kg/day) increases the incidence of Raynaud's disease and cyanosis of fingers and toes. Additionally, arsenic may cause hematological diseases like anemia, leucopenia and thrombocytopenia (Santra et al., 2013) Several metals have been known for a long time to be associated with immune-mediated pathological effects (Becking, 1995).

Vascular diseases (BlackFoot Disease), (Tseng, 2002) and cardiovascular diseases (Navas-Acien et al., 2005) occur from chronic exposure to arsenic. Systemic exposure from the other hand is linked to irritations of the skin and mucous membranes (Sun et al., 2007; Valenzuela et al., 2005). The clinical manifestations of chronic arsenic intoxication are referred to as arsenicosis (hyperpigmentation and keratosis) (Liao et al., 2009). Cumulative prevalence ratios of skin lesions increase with increasing arsenic exposure and age (Liao et al., 2008) for both males and females (Tseng et al., 1968). Quick exposure results in acute

effects characterized by vomiting, abdominal colics, and diarrhea (Caussy, 2003). Neurotoxicity is mainly reported with acute exposure from deliberate poisoning or suicide, or at high concentration in drinking water.

ARSENIC POISONING

Historically, worries over metals have principally been exist due to their acute toxicity. However, as natural and occupational standards become more intense, cases of acute metal toxicity are progressively phenomenal. 50 ppb had been the standard for arsenic in drinking water in United States since 1942. Tseng et al. (Tseng et al., 1968) showed that in area of Taiwan, there were many skin cancers, accompanied by Black Foot Disease. The effected persons were exposed to wells with large amount of arsenic therein. This is perhaps the first public mention that arsenic in water supplies caused these ailments. About this time there were problems noticed in Chile, were unusual number of skin problems in West Bengal were observed. There was large pressure on the EPA to lower the standard for arsenic in water supplies. For over 100 years' toxicologists had depended on rats and mice to give warning about arsenic adverse effects while arsenic widespread pollution continued. After the discovery in Bangladesh and West Bengal, the living laboratories, arsenic has been found in water supplies all over the world. High concentration of arsenic in drinking water have been found in many countries (Asia, Argentina, Taiwan, China, Latin America, Mexico, Greece, Turkey, Finland, Spain, Romania, Hungary, Pakistan, Vietnam), in Bangladesh particularly the number of people suffering from exposure vastly exceeds the number affected by the catastrophic accident at Chernobyl. Arsenic-induced vascular effects have been reported in Chile, Mexico, India, and China, but these effects do not compare in magnitude or severity to Blackfoot disease in Taiwanese populations, indicating other environmental or dietary factors may be involved. Based on the risk of developing cancer from chronic exposure to inorganic arsenic in drinking water, the United States lowered the Maximum Contaminated Levels (MCL) for arsenic from 50 to 10ppb (EPA, 2001).

INTRODUCTION TO INTERNAL DOSE MODELLING

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

PBPK models can describe the mechanisms of absorption, distribution, metabolism and elimination (ADME) of chemicals in the body. These models typically represent the organism as a set of physiological compartments, describing the transport between these, based on physiological processes, such us blood circulation (Nestorov, 2003). PBPK modelling requires several parameters. The parameter set of the PBPK model, includes

anatomical/physiological (cardiac output, tissue blood flow, tissue volumes and weight), physicochemical (partition coefficients), and biochemical parameters (maximal velocity for metabolism: *Vmax*, Michaelis affinity constant: *Km*). Generally, the set is divided in two distinctive subsets: (i) a *drug-specific* subset, which characterizes the pharmacokinetic properties of the particular molecule and is derived from experimental data, and (ii) a *drug-independent* subset, which is derived from the underlying physiological processes.

A PBPK model is derived by compartments. Those compartments correspond different organs or tissues in the body, connected by flow rates (Jones and Rowland-Yeo, 2013). Generally, those compartments include the main tissues of the body: adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, blood, and spleen. Each tissue is typically described as either perfusion rate limited or permeability rate limited (Jones et al., 2006). *Perfusion* rate-limited kinetics tends to occur for small lipophilic molecules where the blood flow to the tissue becomes the limiting process (Rostami-Hodjegan et al., 2012). *Permeability* rate-limited kinetics occurs for larger polar molecules where the permeability across the cell membrane becomes the limiting process. In this case, the tissue is divided into essentially two compartments, representing the intracellular space and the extracellular space which are separated by a cell membrane that acts as a diffusional barrier.

The assessment of potential toxic effects resulting from chemical exposure often involves comparing predicted environmental media concentrations with those known to cause toxic effects. Toxic effects are however the result of internal tissue concentrations, rather than external media concentrations (Cahill et al., 2003). Internal dose refers to the amount of a chemical that reaches the human tissue of interest. This task of estimating concentrations in specific tissues resulting from chemical exposure is addressed by Physiologically Based PharmacoKinetic models. PBPK models are developed in order to organize and describe the available information about the pharmacokinetic processes giving an approximation of the chemical behavior. Based on the available bulk of information, the procedure can be briefly described as: (1) specification of whole body model structure and tissue model structure, (2) equation writing, and (3) parameter specification and/or estimation (Nestorov, 2003).

Those models provide a quantitative mechanistic framework by which chemical-specific parameters can be used to predict the plasma or tissue concentration – time profiles of chemicals (Jones and Rowland-Yeo, 2013; Rostami-Hodjegan, 2012). The major power of a PBPK model is the use of different amounts of administered doses for different exposures scenarios. PBPK modeling of metals requires special considerations as compared to most other chemical species such as drugs and solvents. Metals are often present in multiple media in the ambient environment including groundwater, soil, air. Thus, chronic exposure is an intrinsic part of day-to-day life. Many of the factors that influence the uptake and

disposition of metals differ significantly from those that control the pharmacokinetics of organic compounds (Campain, 2005). Accurate estimation of the risks posed by arsenic exposure to human beings requires effective integration of epidemiological and other human studies in a PBPK model. Through this mechanism we can potentially gain a clearer picture of the relationship between administered and target tissue dose and the resulting toxic effects in humans. To determine the biologically relevant target tissue dose and health effects we must consider the: a) chemical form and bioavailability of Arsenic in environment, including food (Chowdhury et al., 2003; EPA., 1998), b) routes and pathways of exposure (Zartarian et al., 2006).

PBPK models are constructed using a series of differential equations that are parameterized with known physiological variables and represent a quantitative mechanistic framework by which ADME of chemicals can be described in the body. Solving the equations provides the prediction of tissue dose. PBPK model equations are derived from the law of mass action. Once the equations describing the whole body PBPK model, are written and validated, they need to be coded in a particular software language for subsequent estimation and or simulation. In this case study *acslX* http://acslx.com/ from the Aegis Technologies Group, Inc. was used, which is a software environment adept for developing continuous dynamic processes and systems, providing a programming language for the model code, numerical solutions for the ordinary differential equations that define the system being modeled, and a graphical output of the simulations results.

REVIEW OF PBPK MODELS OF ARSENIC

Several authors have worked on a development of a PBPK model for arsenic. The simplest PBPK model for arsenic came from *Yu* (Yu, 1999). This investigator, using short-term oral exposures in rat and mouse, modeled the movement of inorganic arsenic, and did not differentiate between As^{V} and As^{III}. In addition, the metabolism of arsenic through methylation was briefly considered, with MMA and DMA modeled as excreted metabolites whose movement was not accounted for as active arsenic species in the blood or tissue groups. In subsequent work, the model was expanded to more closely fit the human child, while including all arsenic species, and considering both reductive metabolism and methylation. A similar arsenic pharmacokinetic model was described by *Menzel et al.* (Menzel et al., 1994) considering all major forms of arsenic in submodels linked through metabolic processes. This model had several unique aspects, one of which was its steady-state approach to estimating the blood-to-organ ratio of arsenic and its metabolites. The model was never validated.

Mann and co-workers developed a PBPK model for arsenic in hamsters and rabbits (Mann et al., 1996b), which was subsequently scaled to humans (Gentry et al., 2004; Mann et al., 1996a). This model included consideration of inhalation exposure and deposition of As particles in three lung compartments, and diffusion-limited distribution of arsenic to the tissues. The scaled model was tested with experimental data from several studies (Buchet et al., 1981; Vahter and Envall, 1983). Mann et al. suggested that the reduction of As^{V} to As^{III} can be modeled as a first-order oxidation/reduction reaction. Recently, *Gentry* and colleagues extended the model developed by Mann to the mouse (Gentry et al., 2004). These investigators analyzed data from several published studies on experimental arsenic-mediated carcinogenesis in multiple strains of laboratory mice. The ultimate goal of this exercise was to correlate differences in tissue dosimetry and metabolism of arsenic to strain-specific carcinogenic potency of the metal. Although not actually describing development of a true PBPK model, recent work by *Kitchin et al.* links the pharmacokinetics of arsenic to its toxicological effects (Kitchin et al., 1999). These investigators carried out detailed time-course studies in rats on the relationship among administered dose of sodium arsenite, tissue dose of As(i) in the liver and kidney, and the biological endpoint of heme oxygenase induction.

An integrated, biologically based, source-to-dose assessment framework for modeling multimedia/multipathway/multiroute exposures to arsenic was presented by *Georgopoulos* et al (Georgopoulos et al., 2007) where the results indicated that the food intake pathways is the dominant contributor to total exposure and dose to arsenic. Recently, *Stamatelos* et al. (Stamatelos et al., 2011) reported a cellular-level toxicokinetic model which applies in mass action kinetics in order to predict the concentrations of trivalent and pentavalent arsenicals in hepatocytes.

A PBPK model was developed by El-Masri et al. (El-Masri and Kenyon, 2008a) using updated biochemical data, to estimate the levels of arsenic and its metabolites in human tissues and urine after oral exposure to As^{V}, As^{III} or organoarsenical pesticides. The model consists of interconnected individual PBPK models for inorganic arsenic As^{V} and As^{III}, MMA^{V} and DMA^{V}. The inhibitory effects of As^{III} on the methylation of MMA^{III} to DMA, and MMA^{III} on the methylation of As^{III} to MMA were modeled as noncompetitive. Each submodel was constructed using flow limited compartments, which implies that the transport barriers between the free molecules of chemical in blood and tissue are negligible, and equilibration between free and bound fractions in blood and tissue is rapid, describing the mass balance of the chemicals.

METHODOLOGY

ARSENIC PBPK/BBDR MODEL STRUCTURE

PBPK MODEL

The diagram of the PBPK model developed for Arsenic is presented in Fig.4. The model estimates levels of arsenic and its metabolites in tissues and urine after oral and inhalation exposure to either As^{V} or As^{III}. There are two routes of exposure: oral and inhalation and several pathways, such as drinking water, cooking water, food consumption, smoking, breathing. The model, based on El-Masri and Kenyon's model formulation, is composed of four individual PBPK models (see Fig. 4) for As^{V}, As^{III}, MMA and DMA linked together by the transformation of As^{III} to MMA^{V} and DMA^{V}, and the transformation of MMA^{III} to DMA^{V} (methylation). The inhibitory effects of As^{III} on the methylation of MMA^{III} to DMA^{V}, and MMA^{III} on the methylation of As^{III} to MMA^{V} were assumed to follow a non-competitive mechanism. This assumption can be based on studies from the literature (El-Masri and Kenyon, 2008b). Reduction of the pentavalent arsenicals is assumed to follow a first order reaction, because of the ubiquitous availability of thiols such as glutathione in most tissues (Hayakawa et al., 2005).

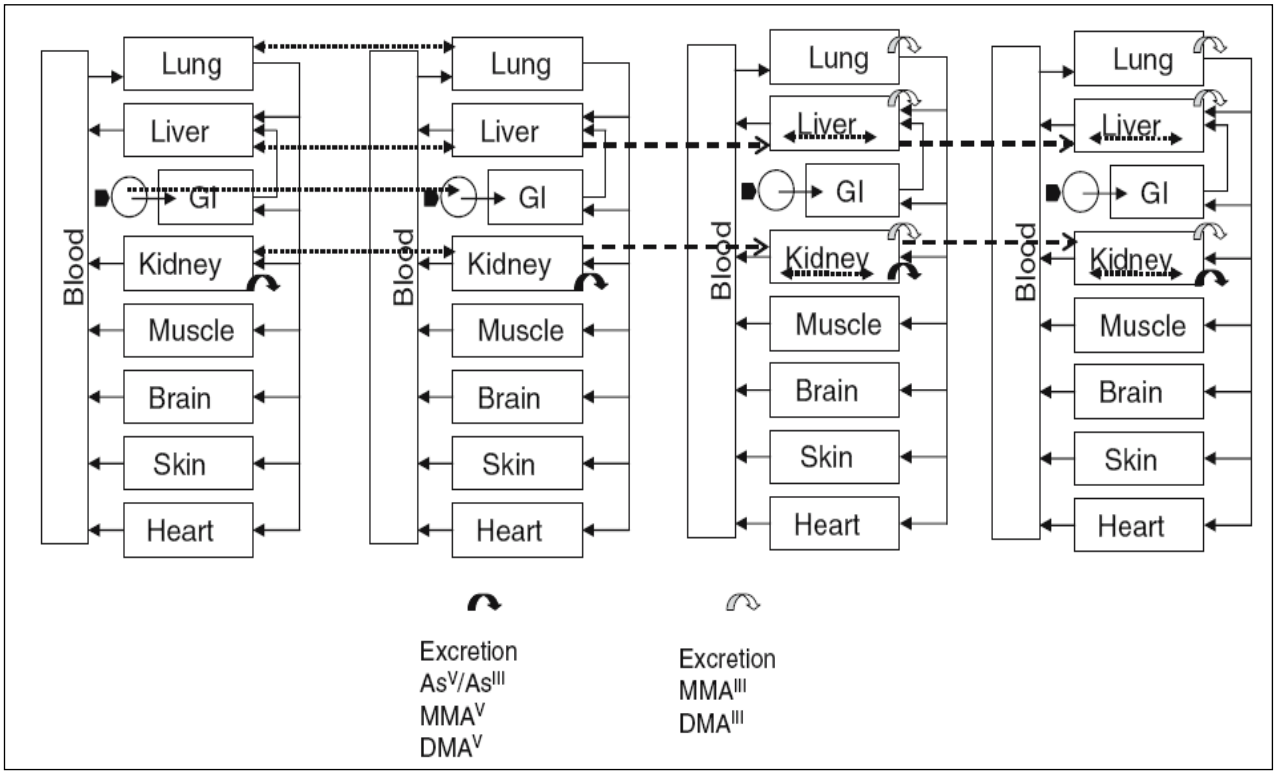

Figure 4. Schematic of the overall PBPK model for inorganic arsenic and methylated metabolites.

Block arrows to the GI tract lumen (circle): oral exposure to As^{III}, As^{V}, MMA^{V} and DMA^{V}; • small dashed line: reduction of As^{V} to As^{III} (GI, lumen, liver, lung and kidney); reduction of MMA^{V} and DMA^{V} to their respective trivalent forms (lung, liver and kidney) oxidation of As^{V}, MMA^{III} and DMA^{III} to their respective pentavalent forms (liver, kidney and lung); • large dashed line: methylation of As^{III} and MMA^{III} (liver and kidney); • curved arrows: urinary excretion.

Reduction is assumed to take place before methylation in the liver, kidney and lung. The oxidation/reduction of inorganic arsenic takes place in the plasma, but the reduction can also occur intracellularly (Cohen et al., 2006; Kumagai and Sumi, 2007; Mann et al., 1996a). The model used Michaelis-Menten kinetics for the methylation of AsIII that takes place mainly in the liver cytosol by enzymatic catalysis and kidney but has also been observed in lung and testes (Georis et al., 1990; Healy et al., 1998; Vahter, 1999). The main methylation pathway in the body is via methionine and its activated form SAM (Vahter, 2000). The model foresees that 90% of the administrate AsV dose is immediately reduced to AsIII in the GI lumen.

In our case, where we need to study the chemical As(i) and its metabolites, the correct approach was a structural variation of a PBPK model for each of the compounds linked together. This situation reflects the fact that metabolism of the liver becomes the input process to the PBPK models of the individual metabolites. Each of the four PBPK model were developed using *flow limited* compartments describing the mass balance of the chemicals in multiple tissues. The tissues have been chosen to account the potential exposure routes (oral, dermal, inhalation), target organs (lung, liver, skin, and kidney) and metabolic sites (liver and kidney) of Arsenic and its metabolites. Brain and heart are also included because they contain significant amount of inorganic arsenic (Benramdane et al., 1999; Saady et al., 1989).The mass balance is expressed as a mathematical equation with appropriate parameters carrying biological significance:

$$V_i \frac{dC_{ij}}{dt} = Q_i\left(CA_j - CV_{ij}\right) - Metab_{ij} - Elim_{ij} + Absorp_{ij} - PrBinding_{ij} \qquad (1)$$

Where: V_i represents the volume of tissue group I, Q_i is the blood flow rate to tissue group i, CA_j is the concentration of chemical j in arterial blood, and C_{ij} and CV_{ij} are the concentrations of chemical j in tissue group I and in the effluent venous blood from tissue I, respectively. $Metab_{ij}$ is the rate of metabolism for chemical j in tissue group i, $Elim_{ij}$ represents the rate of elimination from tissue group i, $Absorp_{ij}$ represents the uptake of the chemical from dosing, and $PrBinding_{ij}$ represents protein binding of the chemical in the tissue.

Once the PBPK model equations are written, their parameters need to be specified and/or estimated. *Physiological and biochemical* parameters are the parameters characterizing the anatomical structure, physiological and biochemical processes of the subject researched. Those parameters are also called *drug-independent* parameters. Among those: bodyweight and tissue/organ/fluid weights and volumes, cardiac output, regional and tissue blood flows etc. *Compound-specific* parameters are parameters characterizing processes such as binding (fraction unbound in blood, plasma or tissues), partition-solubility (blood:plasma ratio or tissue:plasma distribution coefficients) or permeability (permeability surface area products) of the chemical in the various tissues and in blood (Andersen, 1991). Partition coefficients can enable the fate of pollutants in the body, and are a pre-requisite of any PBPK analysis (Abraham et al., 2015).

An understanding of the key ADME mechanisms for a particular compound together with well-defined and measured drug-specific parameter is key to prediction success. Physiological parameters and partition coefficients for each tissue compartment were obtained from the literature (El-Masri and Kenyon, 2008a). The analysis presented in this work does not include inherent interindividual metabolic variability (i.e., all variability is attributed to physiological and activity variation). The numerical values of the physiological and biochemical parameters (El-Masri and Kenyon, 2008a) are reported in Table 2. The metabolic parameters are shown in Table 3.

Table 2. Physiological and biochemical parameters used within the Arsenic PBPK model

Tissue	Tissue Volume (L)	Blood Flow (L/min)	Tissue/Blood Partition Coefficients			
			ASV	ASIII	MMA	DMA
GI	1.2	1	2.7	8.3	2.2	2.1
Skin	2.6	0.26	7.9	7.4	2.61	2.4
Brain	1.4	0.63	2.4	2.4	2.2	3.3
Heart	0.35	0.2	7.9	7.4	2.61	2.4
Kidney	0.28	1	8.3	11.7	4.4	3.8
Liver	1.82	0.31	15.8	16.5	3.3	3.3
Muscle/other	55.5	1.8	2.1	6.7	1.3	1.3
Lung	0.56	5.2	7.9	7.4	2.61	2.4
blood	5.53	-	-	-	-	-

Table 3. Metabolic parameters used within the Arsenic PBPK model

PARAMETER	DESCRIPTION	VALUE	UNITS
DMA			
Ka	Oral absorption	0.007	min^{-1}
Kred	Reduction of DMA	0.004	min^{-1}
Kox	Oxidation of DMA III	0.65	*unitless*
Kurine/DMA	Urine Excretion Const	0.13	min^{-1}
MMA			
Ka	Oral Absorption	0.007	min^{-1}
Kred	Reduction of MMA	0.008	min^{-1}
Kox	Oxidation of MMA III	0.63	*unitless*
Vmax (MMAIII→DMA)	Methylation of MMA III	6.6×10^{-7}	*mole/min*
Km (MMAIII →DMA)		3×10^{-6}	*M*
Kinh	Noncompetitive Const inhibition	4×10^{-5}	*M*
Kurine/MMA	Urine Excretion Const	0.3	min^{-1}
Inorganic Arsenic			
Ka (Asv)	Oral absorption	0.003	*min−1*
Ka (AsIII)		0.004	*min−1*
Kred	Reduction of AsV	0.003	*min−1*
Kox	Oxidation of As III	0.25	*unitless*
Vmax (AsIII →MMA)		5.3×10^{-7}	*mole/min*
Km (AsIII →MMA)	Methylation of As	3×10^{-6}	*M*
Vmax (AsIII →DMA)		2×10^{-6}	*mole/min*
Km (AsIII →DMA)		3×10^{-6}	*M*
Kinh	Noncompetitive inhibition const	4×10^{-5}	*M*
Kurine/As	Urine Excretion Const	0.07	min^{-1}

The model simultaneously evaluates the distribution, conversion, and losses of chemical species. Conventional Michaelis-Menten kinetic parameters are used to determine the reaction rates. Reactions follow first-order kinetics at low concentrations, and a maximum reaction rate is approached at high concentrations. The distribution of the four forms of arsenic within the different organs is described through linear dynamic equations, which can be expressed as in Equation:

$$\frac{d\{A(t)\}}{dt} = [K]\{A(t)\} + [B]\{q(t)\} \quad (2)$$

Where: $\{A(t)\}$ state variable vector which describes the chemical amount in each assigned target organ, $\{q(t)\}$ input vector expressing the dose rate of chemical entering the organisms, [K] state matrix which describes the diffusion exchange rate between target organs and [B] constant input matrix with describes the exchange rate into target organs.

Renal excretion is modeled as a series of processes(Bridges and Zalups, 2005; Buchet et al., 1981). Kidney is the site of urinary excretion for AS^{III}, AS^{V}, MMA^{V}, DMA^{V}, MMA^{III}, DMA^{III}; excretion of MMA^{III} and DMA^{III} occurs also from lung and liver, considering this as an overall estimate of clearance of the chemicals from tissues where they are formed. As a metalloid, arsenic undergoes biotransformation to produce polar methylated metabolites which can be used as substrates for various transporters (El-Masri and Kenyon, 2008a).

Model simulations were tested against available data from other studies using human subjects (Lee 1999). The author investigated the kinetics of inorganic arsenic ingestion in humans. Subjects recruited for this study were three males and two females aged 23–60 years. On the morning of the exposure day, subjects were allowed to consume their normal breakfast. One hour before their noon meal, volunteers were asked to completely empty their bladders. Then they consumed a solution containing 100 μg of sodium arsenate (As^{V}) or sodium arsenite (As^{III}). For the next 12 hours all voided urine was collected. Urine samples were then analyzed for As, MMA, and DMA. The calibrated overall model was evaluated against data provided from these human studies. The results of the model simulation in comparison to data for the arsenite experiment are given in Fig.5. Results show a good agreement between the experimental data and the modeled ones. The PBPK model provides generally better results when it simulates initial dose of As^{V} (right box) rather than of As^{III} (left box). For both the exposures scenarios, among the four Arsenic chemical forms tracked by the model, As^{III}, As^{V} and MMA are predicted with a high level of accuracy both in terms of actual values of and in terms of shape of the curve which is related to the kinetic of arsenic.

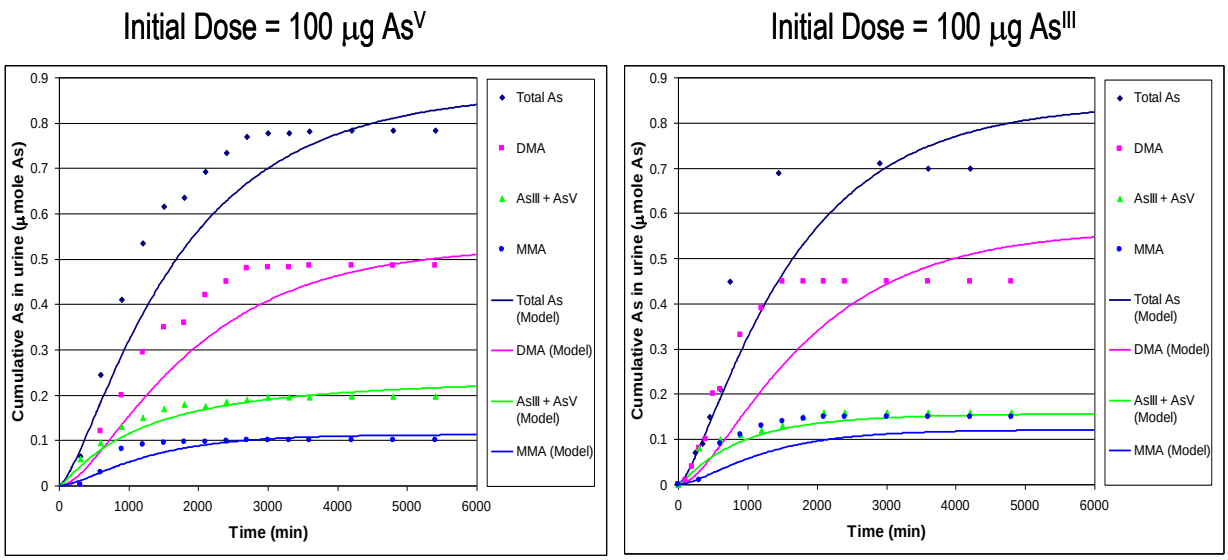

Figure 5. Validation of the Arsenic PBPK model

PBPK model should include those tissues and organs that are essential for the pharmacokinetics of the compound (this means that an adequate model should consists of a fairly large number of tissue/organs). Also, the complexity must be limited for practical reasons (need of data and information, numerical and computation time problems). In this case study, PBPK model is integrated with a Biologically Based Dose-Response approach (PBPK/BBDR) in order to derive internal organ concentrations from external exposure and link these concentrations to the health effects.

BBDR MODEL

Dose-response assessment, is the estimation of the relationship between dose or level of exposure to arsenic, and the incidence of an effect (Leeuwen, 2007). BBDR models provide the substrate for simulations that link mode of action research with predicted physiological consequences of exposures (Andersen et al., 2002). Once the internal doses are calculated via the PBPK model, the next step is to link the internal dose with the health point considered to assess the quantitative risk associated with the given exposure (Clewell et al., 2007; Conolly and Andersen, 1993). The result is a quantitative estimate of health risk relevant to specifics health end-points in the exposed population.

A risk assessment not taking into account the different species but considering only total arsenic, would lead to a considerable overestimation of the health risk related to arsenic exposure (Chain, 2009), therefore it is required to relate the toxicity of all the forms of arsenic found in the PBPK model, to the toxicity of the trivalent arsenic. An in vitro study with human epidermal keratinocytes showed the relative toxicities: $As^{III} > MMA^{III} > DMA^{III} > DMA^{V} > MMA^{V} > As^{V}$ (Vega et al., 2001). Among the different forms in which arsenic can be found,

the most toxic is arsenite, followed by arsenate, then the two organic metabolites. However, more recent studies report that the trivalent form of MMA and DMA are likely to be as biologically active as arsenite. Thus, the toxicity order of Arsenic metabolites may be described as follows: DMA^{III}, $MMA^{III} > As^{III} > As^{V} > DMA^{V}$, $MMA^{V} > TMAO$. In general, the toxicity of pentavalent species is lower than that of trivalent by the order of 10^{-3} to 10^{-4} (Hirano et al., 2004; Vega et al., 2001). This may be explained by the faster uptake rate of As^{III} in endothelial cells (Hirano et al., 2003). .

Table 4.Relative toxicity of arsenic species to trivalent inorganic arsenic.

Arsenic form	**AsIII equivalent mole (for 1 mole of compound)**	**Toxicity**
Arsenite – As(III)	1	1
Arsenate – As(V)	1	1/35
Trivalent Monomethylarsonate MMA(III)	0.605	1
Pentavalent Monomethylarsonate MMA(V)	0.536	1/85
Trivalent Dimethylarsinate DMA(III)	0.620	1
Pentavalent Dimethylarsinate DMA(V)	0.543	1/85

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Steps for using PBPK model estimated Internal Dose in Dose-Response Model for arsenic was (Andersen et al., 2005):

1) Identify toxic effects in people, and determine health endpoints from experimental data associated with arsenic exposure
2) Use an appropriate PBPK model to estimate the internal tissue dose metric for various routes of administration, at various doses, for specific exposure scenarios
3) Development of a dose-response model based on the relationship between internal dose and health points.
4) Estimate the probability of the health risks in humans based on the internal tissue dose calculated during human exposures

Step 3 allows us to develop and parameterize a three-stage model (administered dose-internal dose-cancer probability) for cancer growth that links internal doses to health risk probability. In developing BBDR models it is necessary to evaluate the effect of dose on biological parameters of the model. The effects can be described empirically, as has usually been done, or mechanistically. For the cancer models the stochastic aspect involves some probability of division, death, or mutation that occurs randomly (Andersen et al., 2002). Trying to quantify the relation between dose and response probability, it is useful to

decompose the relation between exposure and health risk probability. In this case one relation links the administered dose to the internal dose of arsenic and its metabolites, the other links internal dose to cancer probability. In probabilistic terms it can be explained as follows (Armitage and Doll, 1954).

The dose-response relation between exposure and risk can be denoted by $(p\|x)$ that indicates that the probability of cancer at time *t* is determined by the history of arsenic exposure *x* up to time *t*. The risk depends on exposure, or else, the history $\{y\}$ of inorganic arsenic in different organs. This situation can be diagrammed as $\{x\}^{\rightarrow}\{y\}^{\rightarrow}\{p\}$. This means $\{x\}$ determines$\{y\}$and $\{y\}$ determines $\{p\}$. Thus, the dose-time-response relation $(p\|x)$ may be written as by $(p\|x) = (p\|y) * (y\|x)$. The $(y\|x)$ component corresponds to the relation of a PBPK model (mapping the exposure dose history $\{x\}$ into time courses $\{y\}$ of inorganic arsenic in different organs) and $(p\|y)$ represents an internal dose-response function. The general curve which better describes such relationship is in the form of Hill equation (Cox and Ricci, 1992):

$$P(y) = 1 - e^{(-by+cy^2+dy^3)} \qquad (3)$$

where: *P(y)* = lifetime probability of the health effect, *y* = biologically effective dose of the toxicant at the target organ (internal dose), *b, c, d* = parameters calculated fitting a multistage model to the experimental dataset.

The most common way for calculating mortality (or any other toxic effect) through a dose-effect relationship, is to relate mortality to the pollutant concentration. The pathology model for arsenic uses two different equations for deriving the prevalence of fatal cancer within a given population. These include the Hill equation and an exponential equation (Ling and Liao, 2007) alternatively to the Hill equation:

$$P = 1 - \exp\left[-\left(a + b \cdot C_{H,i}^{c}\right)\right] \qquad (4)$$ and,

$$P = \frac{P_{MAX} \times C_{H,i}^{n}}{EC_{50,i}^{n} + C_{H,i}^{n}} \qquad (5)$$

Where: P = prevalence of the health effect, P_{MAX} = human maximum prevalence of those exposed to the contaminant, $C_{H,i}^{n}$= internal arsenic concentration in human target organ i (μg/g), $EC_{50,i}^{n}$ = 50% effect concentration (μg/g) of P_{MAX} for target organ, a, b, c = parameters calculated fitting a multistage model to the experimental dataset. , n = Hill coefficient which is a measure of cooperativity, an n>1 represent a sublinear (sigmoidal) response indicating positive cooperatively, and n<1 represent a subpralinear response.

DATASET COLLECTION AND EXPOSURE SCENARIOS

The health effects associated to arsenic vary greatly upon several risk modifiers, such as the dose, the duration, and the route of exposure, as well as age, gender, diet, family traits, lifestyle, and state of health.

Arsenic contaminated areas in Greece

The regions where arsenic is found in Greek groundwater sources are classified in three major categories (Katsoyiannis et al., 2015): (1) The geothermal regions, such as in Chalkidiki and in Aridaia region of Northern Greece, (2) The rivers' alluvial deposits such as those in the basins of Aksios, Nestos and Strymon rivers, and (3) Aquifers, influenced by mineralization, resulting in arsenic mobilization over the centuries.

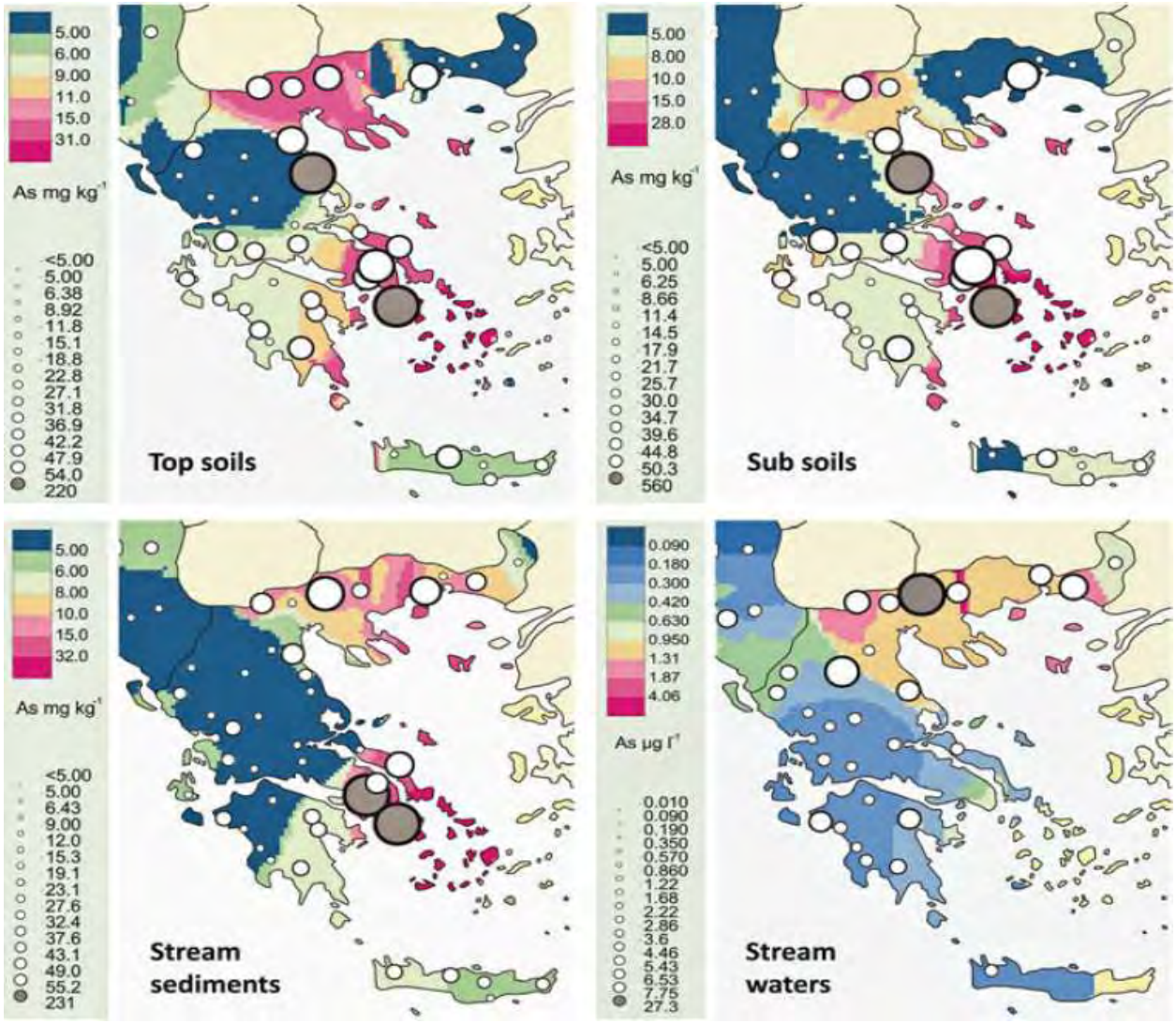

Figure 6 . Arsenic contaminated areas in Greece.

The above figure is a geochemical map of Greece, the lowest As concentrations are represented by the smallest-pen-circle symbol, contracting to the highest As concentrations

that are shown by the biggest-gray-circle symbol. Sources are presumably related to fertilizers, pesticides, municipal wastes, and coal combustion in thermal power plants. Also, there are facts indicating that serious contamination of the environment in Greece, with regard to As, is attributed to combustion of fossil fuels it is believed that the natural (geological) sources of As are equally significant and mainly concern As-containing ores in active and abandoned mining areas, geothermal/hydrothermal waters due to faults (northern Greece) and volcanic activity (southern Greece), coals (mainly lignite in exploited and unexploited deposits), As-minerals in various rock types such as metamorphic rocks, and, certainly, the mineral dust flux derived from Sahara desert. The most important and persisting source of As exposure to the Greek populace appears to be the geothermal and hydrothermal fluids arising from faults as well as the volcanic activity which, in turn, affect underground, surface, and marine aquatic environments (Gamaletsos et al., 2013).

EXPOSURE ESTIMATES OF ARSENIC IN GREECE

Arsenic concentration in water

Concentrations of arsenic in groundwater, are usually less than 10 μg/L (0.1-2 μg/L) but they can reach 5000 μg/L in some areas (Smedley and Kinniburgh, 2002). Drinking water generally contain an average of 2 μg/L of arsenic, although higher levels have been measured (ATSDR, 2007). Surface waters are also used for drinking water, but they generally contain lower arsenic concentrations than groundwaters. The average arsenic content of seawater is about 1.5-1.7 μg/L Several studies have been made across Greece, estimating arsenic concentrations in different media such as groundwater, irrigation wells, drinking water and geothermal waters. A brief review of existing studies is summarized in Table 5.

Table 5. Arsenic concentration in different areas of Greece.

Area	Concentration	Source	Ref.
Petralona, area of Chalkidiki	1.500-2.000μg/L	Geothermal, Irrigation wells	(Katsoyiannis et al., 2015)
Triglia, Central Macedonia	200-400μg/L, As(V)	Geothermal, Irrigation wells	(Katsoyiannis et al., 2015)
Katsiki Mountain and Petralona, area of Chalkidiki	As range:0.001 - 1.840, median: 0.013, mean: 0.311 mg/L, CV:194 *(n=30)*	Groundwater	(Kouras et al., 2007)
Chalkidiki prefecture, Northern Gr.	Water: As: 1000μg/L	Groundwater	(Casentini et al., 2011)
Central Macedonia (Petralona-	Chalastra As range: 180-40μg/L,	Groundwater	(Meladiotis et al., 2002)

Triglia, Therma-Nigrita, Loutraki-Aridea) Northern Gr.	Triglia: 40-160ppb, Chalkidiki and Pellla Prefecture: 80-2000 ppb		
Aksios and Kalikratia areas in N.Gr. 21 locations	As range=: 10–70 μg/L (n-21), As(III)/As(tot) Aksios: 58%, Kalikratia: 10%	Groundwater	(Katsoyiannis et al., 2007b)
Kavala/ Nikisiani	25-30μg/L	Mineralization, Drinking water	(Katsoyiannis et al., 2015)
Kalloni Gulf, Lesvos Island, Polichnitos	Average As: 2.9μg/L	Groundwater	(Aloupi et al., 2009)
Aksios delta/Ghalastra	15-30μg/L As(III)	Drinking water	(Katsoyiannis et al., 2015)
Aksios delta/ Malgara	Total As: 20μg/L, As(III): 14μg/L	Groundwater	(Katsoyiannis et al., 2008)
Aksios delta/Malgara	35-40μg/L,As(III)	Drinking water	(Katsoyiannis et al., 2015)
Aksios delta/Platy	35-45μg/L, As(III)	Drinking water	(Katsoyiannis et al., 2015)
Nestos Delta/ Keramoti	20-27μg/L, As(III)	Drinking water	(Katsoyiannis et al., 2015)
Eastern Thessaly/ Agia & Mpourmoulithra	20-35μg/L, 40-60μg/L	Underground water, Spring water, As(V)	(Katsoyiannis et al., 2015)
Eastern Thessaly region (Sotiritsa, Ano Polydedri)	As range: 1–125, mean: 12, μg/L (n=26)	Groundwater	(Kelepertsis et al., 2006)
Lakes Doirani, Volvi, Koronia	10-80μg/L, Koronia: 13-75μg/L	Lakes deposits	(Katsoyiannis et al., 2015)
Polykastro Kilkis	20-40μg/L	Mineralization, Drinking water	(Katsoyiannis et al., 2015)
Thessaloniki, Industrial area, delta of Axios river	tAs range: 4–130, Median: 6, average: 46 μg/L,	Groundwater	(Katsoyiannis and Katsoyiannis 2006)
Thessaloniki, N-NW area	As median: 5.7, range:1-237μg/L, (n=99)	Groundwater	(Voutsa et al., 1994)
Thessaloniki, Prefecture, agricultural areas	As mean: 2.9, Max: 23.7, Median: 0.1ppb (n=52)	Groundwater	(Fytianos and Christophoridis, 2004)
Greece	As concentration in geothermal water: 30-4,500 μg/L. Regions close to alluvial deposits range: 15-100μg/L. Areas affected by mining activity: 20-60μg/L(Katsoyiannis et al., 2015)		(Katsoyiannis et al., 2015)

Arsenic analyses in various kinds of waters of Greece revealed that its concentration in tap water of 24 big Greek cities did not exceed the new Maximum Contaminant Level (MCL) of 10 μg/L. Moreover, analysis of 125 tap water samples of smaller cities and communities, mainly from Northern Greece, showed that the highest percentage of them (86.4%) complied with the new MCL of 10 μg/L. This percentage was lower (73.6%) in the underground waters used for irrigation. Bottled waters were the least polluted, containing arsenic less than 5 μg/L in general. On the contrary, most of the thermal mineral waters analyzed contained more than 10 μg/L arsenic (Mitrakas, 2001). The ranges of arsenic concentrations in the table above, have been summarized in single concentrations.

Water consumption rate

For estimating the exposure to arsenic from drinking water, a daily consumption rate is needed. A study evaluated the total water intake of Greek Adults in two different groups. Total water intake was 3 L/day in study A; and 2.3 L/day in study B (Athanasatou et al., 2016). Another similar study showed the water intake in general population in Greece was 2.8 l/day in winter and 3.8 l/day in summer (Malisova et al., 2013). A consumption rate of 2 L/day per person was assumed in this study. The water intake varies widely among humans, depending on climate, occupation, and human population, between 2 and 5 l/day (Hough et al., 2010).

Arsenic concentrations in air

Background concentrations of the mean total arsenic concentrations in air range from <1 to 3 ng/m^3, but concentrations in cities may range up to 200 ng/m^3 (ATSDR, 2007).The concentration of As in PM2.5 particles were measured at two sites in the Athens basin (Patission Street and Renis), and industrial area (Thomaidis et al., 2003). The geometric means were 0.79, 0.77, 1.14 ng/m^3 respectively. Seasonal variation indicated that temperature and relative humidity affects positively the concentrations. Another study, collected aerosol samples of PM10 particles during summer and winter from two sites of Messogia Basin, northeast of Athens (Vassilakos et al., 2007). The mean value of arsenic concentration was 14.7 ± 7.3 (range: 8.48-38.1 ng/m^3) and 4.62 ± 2.79 (range: 2.10-11.9 ng/m^3) in Spata and Koropi respectively. Pulmonary exposure may contribute up to approximately 0.14μg/ kg bw/day in a smoker and about 0.01 μg/kg bw/day in a non-smoker, and more in polluted areas. Considering 70 kg b.w. and a daily ventilation volume of 20m^3, the inhaled amount of arsenic would be around 0.001 μg/kg b.w. per day in background situations and up to 0.03 μg/kg b.w. per day in polluted urban areas (EPA, 1984). For Greece, according to HEIMTSA project, the estimated inhaled amount was 0.0028 μg/ kg bw. Regarding the first study conducted above, the range of arsenic in air was 0.0022-0.0033

μg/kg bw, which it compares very well with the inhalation exposure assumed in this study from HEIMTSA project.

Arsenic in contaminated food items

EFSA has created the Comprehensive Food Consumption Database which is a source of information on food consumption across the European Union (EU). It contains detailed data for a number of EU countries. The statistics on food consumption are reported in grams per day (g/day) and grams per day per kg of body weight (g/kg bw per day). Indicative table of food items in EU from the Data Collection and Exposure Unit (DATEX) (EFSA) in in mg/kg Samples from AT: Austria, BE: Belgium, CZ: Czech Republic, DE: Germany, DK: Denmark, EE: Estonia, ES: Spain, FI: Finland, FR: France, GB: Great Britain, HU: Hungary, NO: Norway, PL: Poland, SE: Sweden, SK: Slovak Republic

Table 6. Total arsenic contamination in food items mostly used for consumption across European Union

Food Subgroup	**N**	**<LOD**	**Type**	**P5**	**Median**	**Mean**	**P95**	**Max**	**SAF**
Cereal-based mixed dishes	86	38%	LB	0.0000	0.0029	0.0157	0.0960	0.1640	23%
			UB	0.0014	0.0096	0.0283	0.1133	0.2300	
Cereal grains excluding rice	2215	77%	LB	0.0000	0.0000	0.0147	0.0600	5.6620	22%
			UB	0.0060	0.0262	0.0405	0.0700	5.6620	
Rice grains	1122	9.8%	LB	0.0000	0.1100	0.1362	0.3600	1.1800	4.5%
Cereal products (not specified type)	379	58%	LB	0.0000	0.0000	0.0133	0.0750	0.1800	15%
			UB	0.0050	0.0200	0.0284	0.0750	0.1800	
Cereal products, excluding rice based products	1004	60%	LB	0.0000	0.0000	0.0107	0.0528	0.8900	29%
			UB	0.0030	0.0120	0.0297	0.0750	0.8900	
Rice based products	314	28%	LB	0.0000	0.1000	0.1422	0.3900	1.9800	4.5%
Bran and germ	13	-	LB	0.7100	1.6300	2.1338	6.2400	6.2400	2.0%
Cereals and cereal products excluding dishes	5047	54%	LB	0.0000	0.0000	0.0542	0.2200	6.2400	77%
			UB	0.0050	0.0400	0.0733	0.2250	6.2400	
Chocolate and chocolate based products	558	66%	LB	0.0000	0.0000	0.0125	0.0400	0.3850	33%
			UB	0.0085	0.0200	0.0313	0.0700	0.3850	
Other sugar and sugar products	1403	79%	LB	0.0000	0.0000	0.0140	0.0500	1.0700	67%
			UB	0.0007	0.0120	0.0324	0.0800	1.0700	
Animal fats and oils	142	69%	LB	0.0000	0.0000	0.0075	0.0400	0.1200	23%
			UB	0.0020	0.0100	0.0147	0.0400	0.1200	
Vegetable fats and oils	232	78%	LB	0.0000	0.0000	0.0062	0.0400	0.0990	55%
			UB	0.0050	0.0135	0.0337	0.1000	0.2000	
Butter	254	71%	LB	0.0000	0.0000	0.0055	0.0380	0.0970	22%
			UB	0.0020	0.0080	0.0116	0.0400	0.0970	
Vegetable soups	22	59%	LB	0.0000	0.0000	0.0050	0.0220	0.0260	1.0
			UB	0.0007	0.0045	0.0110	0.0500	0.0500	
Leafy vegetables	1232	58%	LB	0.0000	0.0000	0.0162	0.0560	1.0000	21%
Root vegetables	656	74%	LB	0.0000	0.0000	0.0044	0.0210	0.1280	16%
			UB	0.0030	0.0100	0.0145	0.0400	0.1280	
Stem vegetables	272	89%	LB	0.0000	0.0000	0.0103	0.0500	0.4000	4.0

			UB	0.0030	0.0100	0.0211	0.1000	0.4000	
Peeled potatoes	72	17%	LB	0.0000	0.0015	0.0019	0.0053	0.0073	58.3
			UB	0.0006	0.0015	0.0020	0.0053	0.0073	
Other potatoes	618	85%	LB	0.0000	0.0000	0.0033	0.0160	0.2270	41.7
			UB	0.0017	0.0100	0.0156	0.0500	0.2270	
Berries and small fruits	571	84%	LB	0.0000	0.0000	0.0025	0.0110	0.2900	26%
			UB	0.0020	0.0100	0.0129	0.0250	0.2900	
Other fruits	1763	85%	LB	0.0000	0.0000	0.0063	0.0290	2.1950	70%
			UB	0.0012	0.0100	0.0172	0.0412	2.1950	
Dried fruits	144	71%	LB	0.0000	0.0000	0.0132	0.0550	0.2200	4.0%
			UB	0.0070	0.0210	0.0269	0.0650	0.2200	
Coffee (Powder)	103	67%	LB	0.0000	0.0000	0.0157	0.0740	0.2400	60%
			UB	0.0050	0.0120	0.0235	0.0740	0.2400	
Tea and other infusions	586	54%	LB	0.0000	0.0000	0.0595	0.2700	1.4400	26%
(Powder or dry leaves)			UB	0.0005	0.0105	0.0666	0.2700	1.4400	
Cocoa (Powder or cocoa	245	50%	LB	0.0000	0.0100	0.0409	0.1550	0.8300	14%
bean)			UB	0.0100	0.0500	0.0683	0.1550	0.8300	
Coffee, tea, cocoa	17	5.9%	LB	0.0000	0.0013	0.0044	0.0400	0.0400	-%
expressed as liquid			UB	0.0005	0.0013	0.0044	0.0400	0.0400	
Beer and substitutes	602	72%	LB	0.0000	0.0000	0.0054	0.0180	0.4500	79%
			UB	0.0010	0.0080	0.0161	0.0780	0.4500	
Wine and substitutes	1006	50%	LB	0.0000	0.0010	0.0061	0.0220	0.1110	20%
			UB	0.0023	0.0083	0.0110	0.0240	0.1110	
Other alcoholic beverages	249	49%	LB	0.0000	0.0002	0.0085	0.0200	0.6860	1.0%
and substitutes			UB	0.0002	0.0050	0.0115	0.0300	0.6860	
Bovine, sheep and goat	2102	77%	LB	0.0000	0.0000	0.0039	0.0220	0.0990	20%
meat			UB	0.0020	0.0100	0.0137	0.0300	0.2000	
Pig meat	2013	81%	LB	0.0000	0.0000	0.0037	0.0200	0.1000	42%
			UB	0.0030	0.0090	0.0128	0.0500	0.1000	
Poultry meat	2099	73%	LB	0.0000	0.0000	0.0050	0.0240	0.9800	12%
			UB	0.0030	0.0100	0.0137	0.0400	0.9800	
Other meat	504	58%	LB	0.0000	0.0000	0.0077	0.0420	0.1600	0.20
			UB	0.0028	0.0080	0.0141	0.0450	0.2000	
Processed meat products	1721	68%	LB	0.0000	0.0000	0.0051	0.0230	0.1510	16%
			UB	0.0030	0.0100	0.0162	0.0600	0.1510	
Bivalve molluscs	664	0.30	LB	0.8800	2.4044	3.4075	7.7610	150.00	0.10
			UB	0.8800	2.4044	3.4078	7.7610	150.00	
Cephalopods	189	1.1%	LB	0.0540	1.1000	3.9223	14.600	66.800	3.0
			UB	0.0560	1.1000	3.9232	14.600	66.800	
Crustaceans	344	2.0%	LB	0.1180	2.0290	5.6907	26.000	100.40	0.10
			UB	0.1180	2.0290	5.6910	26.000	100.40	
Other seafood and seafood	150	11%	LB	0.0000	1.5950	11.922	45.300	68.797	0.80
products			UB	0.0030	1.5950	11.923	45.300	68.797	
Seafood and seafood	1347	2.0%	LB	0.0540	2.2000	5.0111	21.270	150.00	4.0
products			UB	0.0590	2.2000	5.0115	21.270	150.00	
Fish and fish products	3503	8.3%	LB	0.0000	0.5800	1.4526	5.0275	195.00	95%
			UB	0.0100	0.5800	1.4549	5.0275	195.00	
Fish based preparations	233	9.9%	LB	0.0000	0.5810	1.1524	4.0700	20.170	1.0

			UB	0.0230	0.5810	1.1573	4.0700	20.170	
Total for Fish and seafood	5083	6.7%	LB	0.0000	0.8400	2.3818	9.8880	195.00	100
			UB	0.0120	0.8400	2.3837	9.8880	195.00	
Total for Eggs	140	76%	LB	0.0000	0.0000	0.0042	0.0240	0.1820	100
			UB	0.0020	0.0100	0.0117	0.0300	0.1820	
Milk and dairy drinks	2366	84%	LB	0.0000	0.0000	0.0026	0.0150	0.1660	57%
			UB	0.0013	0.0080	0.0104	0.0300	0.1660	
Dairy based products	693	77%	LB	0.0000	0.0000	0.0068	0.0120	0.6600	30%
			UB	0.0025	0.0090	0.0184	0.0600	0.6600	
Cheese	837	78%	LB	0.0000	0.0000	0.0065	0.0400	0.2400	13%
			UB	0.0030	0.0100	0.0188	0.0600	0.2400	
Total for Tap water	153	75%	LB	0.0000	0.0000	0.0013	0.0060	0.4700	100
			UB	0.0002	0.0010	0.0022	0.0062	0.4700	

N: number of samples; LOD: limit of detection; LB: lower bound; UB: upper bound; P5: 5th percentile; P95: 95th percentile; Max: maximum; SAF: sampling adjustment factor

The European Commission Scientific Cooperation project found that total arsenic concentrations in most foods other than fish, seafood and rice were in the low range of 0.0005 to 0.020 mg/kg; exceptions were dry tea and coffee powder (0.144 mg/kg), salt and spices (0.097-0.219 mg/kg) and food supplements such as algae preparations (2-42 mg/kg) (all expressed on a dry mass basis). The average total arsenic concentrations in a mix of marine and freshwater fish and other seafood ranged from 0.100 to 1.8 mg/kg. The high concentration of total arsenic in shrimp has been recognized since the beginning of the 20th century (Chapman, 1926). Concentrations of inorganic arsenic were low in all the Atlantic cod analyzed (<0.001 mg/kg), even in fish with high concentrations of total arsenic (Sloth et al., 2005). Tuna was the only fish species with concentration of inorganic arsenic higher than 0.001 mg/kg (i.e. 0.008 mg/kg, total arsenic 0.9 mg/kg). The concentrations of inorganic arsenic in shrimp were <0.001 mg/kg for all samples analyzed. The highest levels of inorganic arsenic were found in crustaceans and with concentrations in blue mussels ranging from 0.001 to 4.5 mg/kg. The percentage of inorganic arsenic to total arsenic in fish fillets for about 20 species caught in the open sea off the Norwegian coast was 0.1 % (except for tuna fish which was about 9 %), and for blue mussels the percentage was on average 1 %.

The main contributor to dietary exposure to As(i) is the food group "*Grain-based processed products*". Other important contributors are rice, milk and drinking water (Authority, 2014). Officials studies for the estimated human exposure to inorganic arsenic in Greece are not available. However, data on the average diet for rice food products in Greece have been delivered by a European project called DAFNE (Data Food Network) it was found that the citizens consume about 16g of grain-based processed products (main contributor to

exposure of inorganic arsenic) were consumed per person per day, putting rice in the list as the top consumed foods in Greece (Pasias et al., 2013).

In contrast, terrestrial foods often have a higher proportion of inorganic arsenic due to contaminated groundwater. In a UK study, total arsenic concentrations in pure baby rice ranged from 0.120 to 0.470 mg/kg with a median of 0.220 mg/kg while inorganic arsenic levels ranged from 0.060 to 0.160 mg/kg, with a median of 0.110 mg/kg. The percentage of inorganic to total arsenic ranged from 33 % to 68 % with a median of 57 %. In a Swedish study, the mean concentration of total arsenic in long grain brown rice of 0.240 mg/kg was similar to that of parboiled white rice at 0.210 mg/kg, whereas white rice contained considerably less arsenic (0.100 mg/kg). The concentration of inorganic arsenic averaged 0.110 mg/kg, or 64 % of the total arsenic (Jorhem et al., 2008). Some common food items (bread, rice, milk, pork meat, chicken meat, cabbage and potatoes) from the Slovak Republic were collected and analyzed for total arsenic concentrations. Rice contained the highest average concentration of arsenic of 0.158 mg/kg. The major proportion of the arsenic in rice seemed to be inorganic. Also, potatoes at 0.033 mg/kg and poultry meat at 0.028 mg/kg contributed to arsenic exposure, although arsenobetaine accounted for more than 80 % in the poultry meat. When the potatoes were peeled the concentrations of arsenic were lowered to 0.0023 mg/kg.

Arsenic concentration in soil

Arsenic in soil could be derived from both natural and anthropogenic sources. Atmospheric pollution and application of phosphate fertilizers appear to be major contributors to the anthropogenic arsenic deposition in agricultural soils. Atmospheric deposition of arsenic into soil has generally decreased over the last 20 years in Europe (DG Environment, 2000). Background arsenic levels in surface soils range from 0.1 to 55 mg/kg, with mean values often around 5 mg/kg (Matschullat, 2000). Mean sediment arsenic concentrations may range from 5 to 3000 mg/kg. The concentration of arsenic in forest soil samples influenced by industrial activities were reported to range from 120 to 252 mg/kg dry mass.

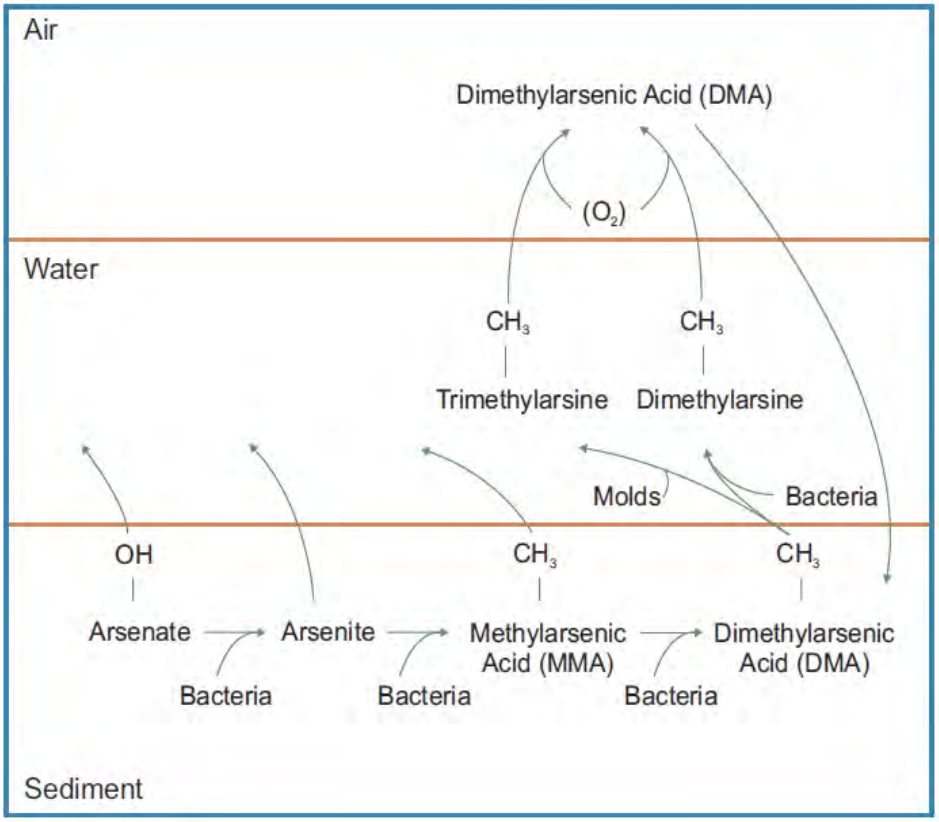

Figure 7. Different forms of Arsenic in the various environmental compartments

Assessment of multimedia transfer

As discussed in Chapter 1. arsenic ends up in food, plants and tap water, depending from the geochemical conditions, and from groundwater in a similar way. In order to estimate the arsenic contamination in food and tap water, we must calculate the amount transferred, (multimedia transfer) by assuming that food and tap water will be contaminated in a similar way from the concentration found in the groundwater in that specific area. This was done by the environmental fate model WATSON in the Health and Environment Integrated Methodology and Toolbox for Scenario Assessment (HEIMTSA) project. Using the concentration of arsenic in groundwaters, given in the Table 5. we can estimate the food and tap contamination of those areas to assess the total oral intake form dietary (food) and non-dietary (water) pathways. Data are obtained from the Health and Environment Integrated Methodology and Toolbox for Scenario Assessment (HEIMTSA) project. In this project Integrated Water and Soil Environmental Fate, Exposure and Impact Assessment Model of Noxious Substances in Europe (WATSON) model was used to estimate both concentration in terrestrial and aquatic environmental median as well as human exposure though ingestion of various food items and through drinking water. In addition, exposure via inhalation were calculated on the base of concentration in air in each capita.

In general, the concentration in soil and rice (the major contributor to arsenic dietary exposure) and various crops (both in mg/kg) is associated with the following formula (Santra et al., 2013):

$$C_{rice} = C_{soil} * 0.025 \quad (6)$$

$$C_{vegetables} = C_{soil} * 0.005 - 0.05 \text{ varying for the different types of crops} \quad (7)$$

Uptake of As by plants occurs primarily through the root system. Because arsenic is not readily translocated to the shoots, the edible plant parts are generally low in As (<2 mg kg). Because As is highly toxic to plants at concentrations that do not yet affect animal or human health, crop damage or even failure is usually assumed to occur before As levels in shoots are of concern for animal or human health (Gulz et al., 2005). Tuberous vegetables accumulate higher amount of arsenic than leafy vegetables and leafy vegetables followed by fruity vegetable. The highest arsenic accumulation is observed in potato, brinjal, arum, amaranth, radish, lady's finger, cauliflower whereas lower level of arsenic accumulation is observed in beans, green chili, tomato, bitter guard, lemon and turmeric. The root, shoot and leaf tissue of rice plant contain mainly inorganic AsIII and AsV while the rice grain contains predominantly DMA (85 to 94%) and AsIII. Using the above information, we can estimate the food concentration to Arsenic in the Mediterranean diet.

Table 7. Time-integrated accumulated country-specific concentrations of arsenic in fresh water and fresh water sediment as output of the environmental fate module of the WATSON model for scenarios in the year 2010 including direct and indirect releases into fresh water and agricultural soils [µg $_{As}$/L $_{water}$ or µg $_{As}$/kg $_{Sediment\ (dry\ weight)}$].

	HEIMTSA 2010 BAU	
Country	Freshwater Body	Freshwater Sediment
Greece	1.27	30.53

Table 8. Time-integrated accumulated country-specific concentrations of arsenic in different terrestrial compartments as output of the environmental fate module of the WATSON model for scenarios in the year 2010 including direct and indirect releases into fresh water and agricultural soils [mg $_{As}$/kg $_{soil\ (dry\ weight)}$].

	HEIMTSA 2010 BAU			
Country	arable land (unspecified)	non-vegetated soil/rock	pasture grassland	(semi-)natural ecosystems
Greece	1.86	1.19	1.93	4.44

Overall, the steps for estimating daily intake for a given area in Greece include:

1) The information based on EFSA's dietary survey for calculating the amounts of different food items consumed in Greece per day,

2) The estimated of WATSON model, for assessing As multimedia environmental fate to estimate the contamination of food items, plants and tap water, starting from known arsenic levels groundwater in different areas in Greece. In cases where only the arsenic concentration in drinking water was known a mean value of 40μg/L total arsenic in groundwater was used to estimate the residues in food items

Accounting for all the above, the complete picture of dietary and non-dietary arsenic intake was formed for several areas in Greece.

Table 9. Total arsenic intake from food, water ingestion and inhalation in specific areas in Greece using known levels of Arsenic in drinking water. Using the environmental fate module of the WATSON model combined with known concentrations of arsenic in groundwater we obtain different exposure concentration in food (shaded blocks).

Area	Source	Levels of As μg/L	Tap water μg/kg bw	Food μg/kg bw	Inhalation μg/kg bw	Total intake μg/kg bw
Petralona	Irrigation wells	1.500	30.82	1.49	0.0028	32.32
Chalkidiki prefecture, N.Gr.	Groundwater	1000	0.059	12.21	0.0028	12.27
Triglia	Irrigation wells	200	4.11	1.4	0.0028	5.63
Island of Kos	Drinking water		4.11	1.49	0.0028	5.63
Thessaloniki NW.Gr.	Groundwater		0.05	3.23	0.0028	3.29
Aksios, Kalikratia	Groundwater	50	0.05	1.55	0.0028	1.61
Thessaloniki, delta of Aksios	Groundwater	46	0.05	1.50	0.0028	1.56
Aksios delta, Malgara, Ghalastra, Platy, Keramote, Kavala, Nikisiani, Polykastro Kilkis	Drinking water	30	0.61	1.49	0.0028	2.11
Kavala, Nikisiani, Nestos Delta/Keramoti	Drinking water	25	0.51	1.49	0.0028	2.02
Serres	Drinking water	20	0.41	1.49	0.0028	1.93
	Groundwater	10	0.20	1.49	0.0028	1.76
Eastern thessaly, Lesvos Island		12	0.05	1.12	0.0028	1.18
Kalloni Gulf, Lesvos Island	Groundwater	2.9	0.05	1.02	0.0028	1.08

EXPOSURE ESTIMATES STARTING FROM BIOMONITORING DATA

Biomonitoring is a commonly used practice for assessing human exposure environmental contaminants, and arsenic is one of the most commonly biomonitored heavy metal. Samples of hair, nails, urine, saliva, sweat or blood are collected and analyzed for Arsenic compounds and their metabolites. Levels of arsenic or its metabolites are used as biomarkers of arsenic exposure. Biomarkers present a time-variable concentration profile associated with temporal patterns of exposure and elimination kinetics (WHO, 2015).

The arsenic metabolites are excreted mainly in urine (El-Masri and Kenyon, 2008a) with concentrations generally ranging from 5 to 20 μg/L, but may exceed 1000 μg/L (Waseem and Arshad, 2016). The concentration of metabolites of inorganic arsenic in urine (MMA, DMA) reflects the absorbed dose of inorganic arsenic on an individual level (WHO. et al., 2001). In humans, the relative proportions of As species in the urine are usually about 10-30% As(i), 10-20% MMA, and 60-70% DMA (Orloff et al., 2009). Speciated metabolites in urine expressed either as inorganic arsenic or as the sum of metabolites [*As(i)+ MMA + DMA*] provide the best quantitative estimate of recently absorbed dose of arsenic. Urine is the most frequently used biological medium for biomonitoring. Urine is a readily, easily collected with good reference range sample matrix which is accessible in large volumes. One can monitor the drug in the urine in order to obtain selected pharmacokinetic parameters of a drug as well as other useful information such as the bioavailability of a drug. There is a direct proportional relationship between the observed plasma concentration and/or the amount of drug eliminated in the urine and the exposure dose of a chemical. Measuring the urinary concentration of Arsenic is useful in assessing recent exposure to Arsenic, and high-quality reference ranges are available for urinary Arsenic concentrations. Normal human levels of arsenic are <1μg/L in blood, <100μg/L in urine and ≤ 1 ppm in nails and hair (ATSDR, 2007). Blood arsenic is a useful biomarker in the case of stable chronic high-level exposure but Arsenic is rapidly cleared from blood, and speciation of its chemical forms in blood is difficult. Arsenic in hair and nails can be indicators of past arsenic exposure, provided care is taken to prevent external arsenic contamination of the samples. Arsenic in hair may also be used to estimate relative length of time since an acute exposure. The reference range for arsenic in human hair lies in the range of 0.0003–0.34 μg/g.

PBPK models are powerful tools and can be used additionally to estimate the biomonitoring equivalent levels (BEs). Those levels, represents the concentration of the parent chemical or its metabolite, in a biological sample, and is consistent with the established reference values for intake levels (WHO, 2015). The BE value for arsenic is

6.4μg/L and the intake-based reference value is 0.3μg/kg/day. PBPK model, gives us the opportunity from limited data for human excretion of arsenicals in urine, to be used to estimate the exposure. In case of Greece, biomonitoring data for arsenic are missing. In Greece currently there is no established human biomonitoring system organized by the competent and local/regional authorities for public health protection. A Project called: Cross-Mediterranean Environment and Health Network – CHROME-LIFE (http://www.crome-life.eu/) will allow researchers to assess different levels of environmental exposure, age windows, socioeconomic and genetic variability in four demonstration sites, including Greece.

The exposure conversion factor (ECF) method, proposed by Tan et al. (2006), assumed that the relationship between biomarker and dose can be approximated by a linear function for exposure reconstruction purposes. This approach involves three steps: (1) generating samples for forward model runs from distributions of possible exposure, physiological, and biochemical parameters, (2) running the forward model using a set of input samples from these distributions, and (3) inverting the distribution of output (i.e. simulated biomarker levels) to obtain an ‘‘ECF.’’ Using the ECF and the distribution of observed biomarkers, the possible exposures for that particular biomarker distribution can then be estimated through a straightforward convolution (Tan et al., 2006). In a typical application of this simple method, the PBTK model can be run using a unit dose or concentration value, and various samples from the possible distributions of parameters such as activities, physiological parameters, biochemical parameters, biomarker sample times, etc., to generate a set of biomarker levels. These levels then provide the distribution of biomarkers for a unit exposure metric, which can be inverted to obtain an ECF in units of the exposure metric divided by biomarker level units. The ECF can then be multiplied by the values of available biomonitoring data (e.g. from biomarker databases such as NHEXAS or NHANES) to produce an estimate of dose distributions for the corresponding population. This convolution is performed by multiplying samples from the biomarker distribution with samples from the ECF distribution. The aggregate samples then provide the distribution of reconstructed exposures. Though this method is conceptually simple and straightforward to use, as it involves direct generation of samples of the corresponding statistics from these samples, the ECF can be highly sensitive to the assumptions of the prior distributions. Furthermore, the assumption of linearity can sometimes produce unreasonably large tails in the distribution of reconstructed exposure metrics, especially when exposures occur infrequently, and the sampling time relative to the last exposure is unknown.

Table 10. Biomonitoring studies around the world using Arsenic biomarkers in urine. Those studies were used to calculate the external intake dose by using the exposure conversion factor to calculate the individual risk and health impact to fatal cancer.

Study design	***Sample Type***	***As concentration (μg/L)***	***Calculated Intake dose using ECF, mole/min***	***Calculated Intake dose using ECF, μg/kg bw***	***Ref***
Concentration of various toxic elements	Urine	As: Median: 12.5 range: 4.8-200	2.0147E-11	0.31	(Genuis et al., 2011)
Location: Germany region with As in soil Participants: n= 218 *(North) and* n=76 *(South) nonexposed subjects Aim: determination of the internal exposure to As*	Urine	North: Median: 3.21, range: 0.1-18.32 South: Median: 6.20 range:0.29-23.8	North:5.17374E-12 South:9.9929E-12	North: 0.08 South: 0.15	(Gebel et al., 1998)
Male diabetics and normal subjects, Hyderabad, Sindh, Pakistan	Urine	Non-smokers control: 3.39–5.61(4.7) Smoker control: 4.88 –5.96 (5.41)	Non-smokers control: 7.57527E-12 Smoker control 8.71961E-12	Non-smokers control: 0.12 Smoker control 0.13	(Waseem and Arshad, 2016)
Adults selected from various countries in Pakinstan	Urine	Mean = 20 Range 10-30	3.22352E-11	0.50	
French National Survey on Nutrition and Health 2006-7 (adults)	Urine	Mean = 11.96	1.92766E-11	0.30	
Non Occupationally exposed adults, Belgium	Urine	Mean = 15.4	2.48211E-11	0.38	(Waseem and Arshad, 2016)

Occupationally unexposed UK adults	Urine	Median = 10.48	1.68912E-11	0.26	(Waseem and Arshad, 2016)
Flemish Human Biomonitoring Program (FLEHS II: 2007-11), Belgium, Females	Urine	Geometric Median: 17.2	2.77222E-11	0.43	
USA (NHNES) 2011-15	Urine	Geometric Median: 6.85	1.10405E-11	0.17	
Canadian Health Measures Survey Cycle 2&3, 2009-13	Urine	Mean: 9.2	1.48282E-11	0.23	
Aim: Relationship of exposure, age, group, city factors with urinary arsenic Location: Chile, n=756	Urine	Antofagasta: Students Median: 49.8 Santiago: Students mean: 27.8 Temuco: Students mean: 17.2	Antofagasta**:** 8.02656E-11 Santiago 4.48069E-11 Temuco 2.77222E-11	Antofagasta: 1.24 Santiago 0.69 Temuco 0.43	(Caceres et al., 2005)
*Location: Winkelman and Hayden (Arizona) Aim: relationship of arsenic in house dust to inorganic urinary arsenic (*n=404*)*	Urine	Total Arsenic (Hayden): 14.4, Total Inor. Arsenic: 12.6 Total Arsenic (Winkelman): 12.3 Total Inor. Arsenic: 11.7	Hayden 2.32093E-11 Winkelman 1.98246E-11	Hayden 0.36 Winkelman 0.31	(Hysong et al., 2003)
Study: Excretion of As among adults in urine after 24h water intake Location: Yaqui Valley, Sonora, Mexico, July 2001-May 2002.	Urine	Total As: 64.5μg/L (Esperanza) 29.5(Cocorit) 38.4 (Pueblo Yaqui)	Esperanza 1.03958E-10 Cocorit 4.75469E-11 Pueblo Yaqui	Esperanza 1.60 Cocorit 0.73 Pueblo Yaqui	(Meza et al., 2004)

		6.18915E-11	0.95		
		As(III): 14 (Esperanza), 3.3(Cocorit), 6.3 (Pueblo Yaqui)			
		As(V): 1.7 (Esperanza), 1.3(Cocorit), 1.6 (Pueblo Yaqui)			
		MMA(V): 6.3 (Esperanza), 4.2(Cocorit), 2.9 (Pueblo Yaqui)			
		DMA(V): 34.6 (Esperanza), 18.8(Cocorit), 17.3 (Pueblo Yaqui)			
Aim: Comparison of arsenic concentrations species of non-exposed and exposed subjects to As, after consuming fish Location: U.K.	Urine	As(III): Control: 0.4, Exposed: 0.6 (n=9)	Control: 1.36999E-11 Exposed: 4.86751E-11	Control: 0.21 Exposed: 0.75	(Morton and Mason, 2006)
		DMA: controls: 4.6, exposed: 7.3, (n=34) MMA: controls: 0.7, exposed: 0.5 (n=34) As(V): controls: 2.8, exposed: 3 (n=9)			
Aim: pattern of excretion of arsenic compounds in urine to adults in exposed area Location: Bangladesh	Urine	Total arsenic: 175.7 exposed to contaminated water 300		2.83186E-10	4.36 (Chowdhury et al., 2003)
		Total arsenic: 385.5 exposed to contaminated water 340		6.21333E-10	9.58
		Total arsenic: 560.2 exposed to contaminated water 540		9.02907E-10	13.92
		Total arsenic: 494.7 exposed to contaminated water 460		7.97337E-10	12.29
Study: Urine samples (3000) from residents of a community surrounding an arsenic-emitting copper smelter	Urine	Arsenite: 20+y: Males: 2.1, Females: 1.2			(Kalman et al., 1990)
		MMA: 20+y: Males: 2.3, Females: 1.7	Males: 2.09529E-11, Females: 1.49894E-11	Males: 0.32 Females 0.23	
		DMA: 20+y: Males: 6.4, Females: 6.4			
		Sum: 20+y: Males: 13, Females: 9.3			

RESULTS

EXPOSURE SCENARIO IN GREECE

The water amount drunk per day, food consumption and the amount inhaled are inputs to the PBPK model as ingestion or inhalation rates (mole/min). This has allowed the calculation of internal concentrations (Table 10) which cause the fatal cancer individual probability reported in figures 8, 9, 10, 11. The values of individuals were then multiplied by the total population resulting in the estimated overall number of deaths attributable to the specific cancer on country basis.

Table 11. Internal concentrations obtained from the simulations run in the PBPK model, after reaching a steady-state condition, for each individual case with different amounts of contamination either in groundwater or drinking water. The internal concentration is the amount of total arsenic, inorganic and organic species after having transformed to the equivalent toxicity compared to Arsenite.

Arsenic contamination level (μg/L)	**Internal Concentration (mg/L)**			
	Lung	**Kidney**	**Liver**	**Skin**
	Vl_{ung}=0.56	V_{kidney}=0.28	V_{liver}=1.82	V_{skin}=2.6
(Groundwater) 2.9	6.9E-05	2.1E-04	6.6E-05	1.6E-05
(Groundwater) 12	7.7E-05	2.2E-04	7.2E-05	2.3E-05
(Tap water) 10	7.5E-05	2.4E-04	7.2E-05	1.8E-05
(Tap water) 20	8.4E-05	2.6E-04	8.1E-05	1.9E-05
(Tap water) 25	8.9E-05	2.6E-04	8.6E-05	2.1E-05
(Tap water) 30	9.3E-05	2.8E-04	9E-05	2.2E-05
(Groundwater) 46	1.2E-04	3.2E-04	1.2E-04	3.2E-05
(Groundwater) 50	1.7E-04	3.2E-04	1.3E-04	3.5E-05
(Tap water) 200	2.3E-04	6.5E-04	2.4E-04	6.3E-05
(Groundwater)1000	1.1E-03	2.3E-03	9.6E-04	2.7E-04
(Groundwater)1500	1.6E-03	3.1E-03	1.7E-03	3.9E-04

Once the internal concentrations are known, linking those to the specific health event probabilities will allow obtaining the individual risk and the health impact of the population to fatal cancer studied.

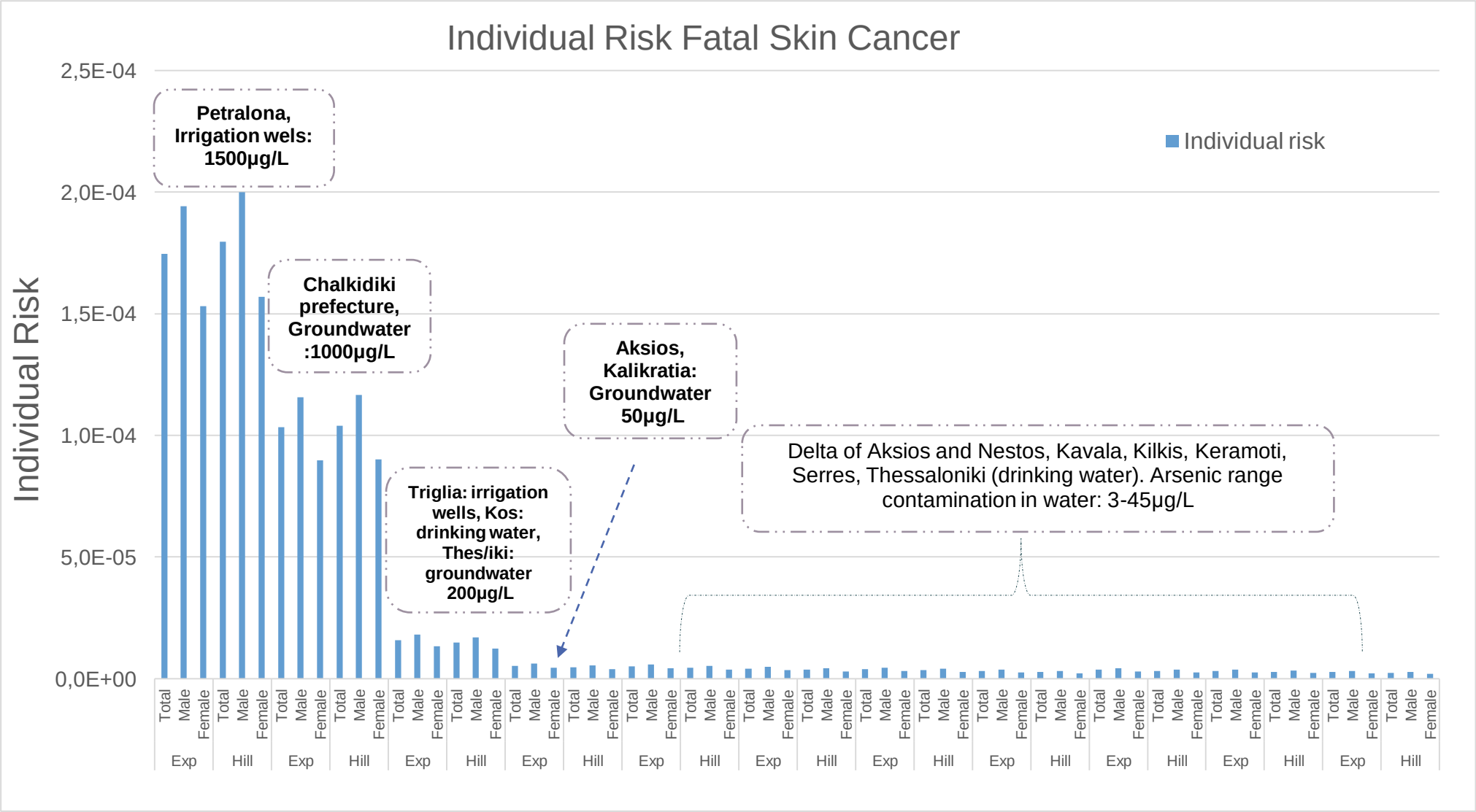

Figure 8.Individual Risk of Fatal Skin Cancer to several areas in Greece.

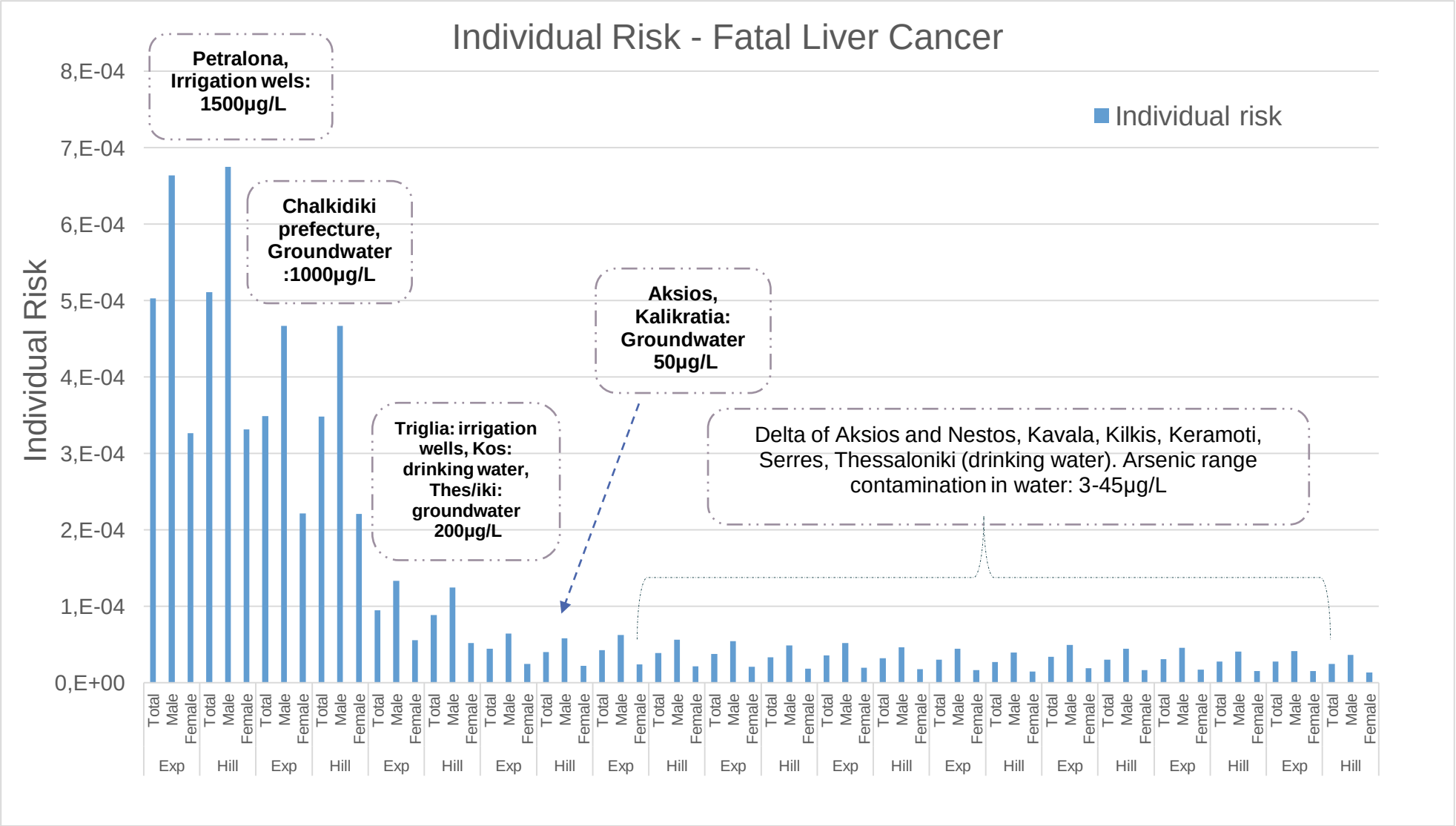

Figure 9. Individual Risk of Fatal Liver Cancer to several areas in Greece.

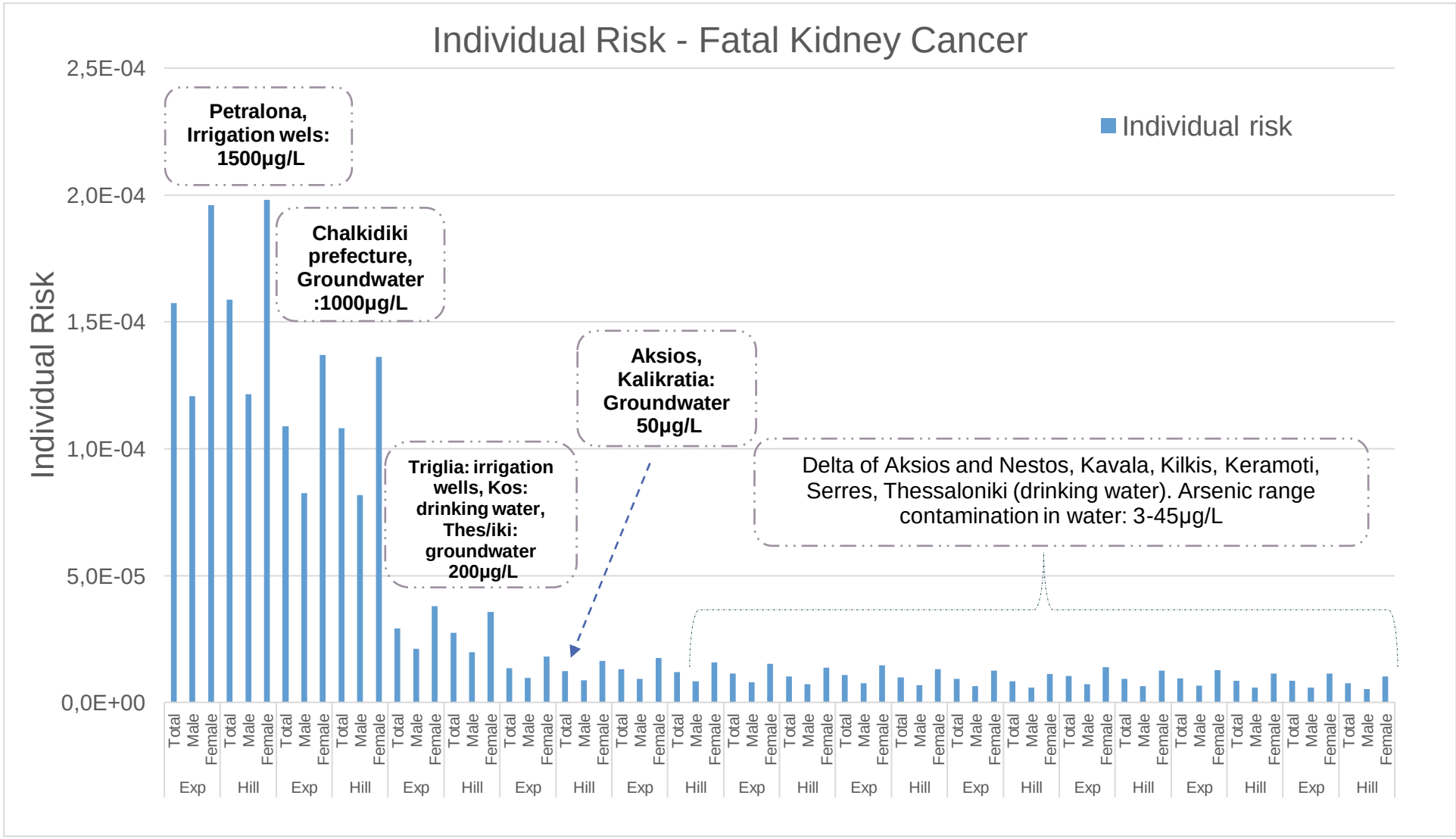

Figure 10. Individual Risk of Fatal Kidney Cancer to several areas in Greece.

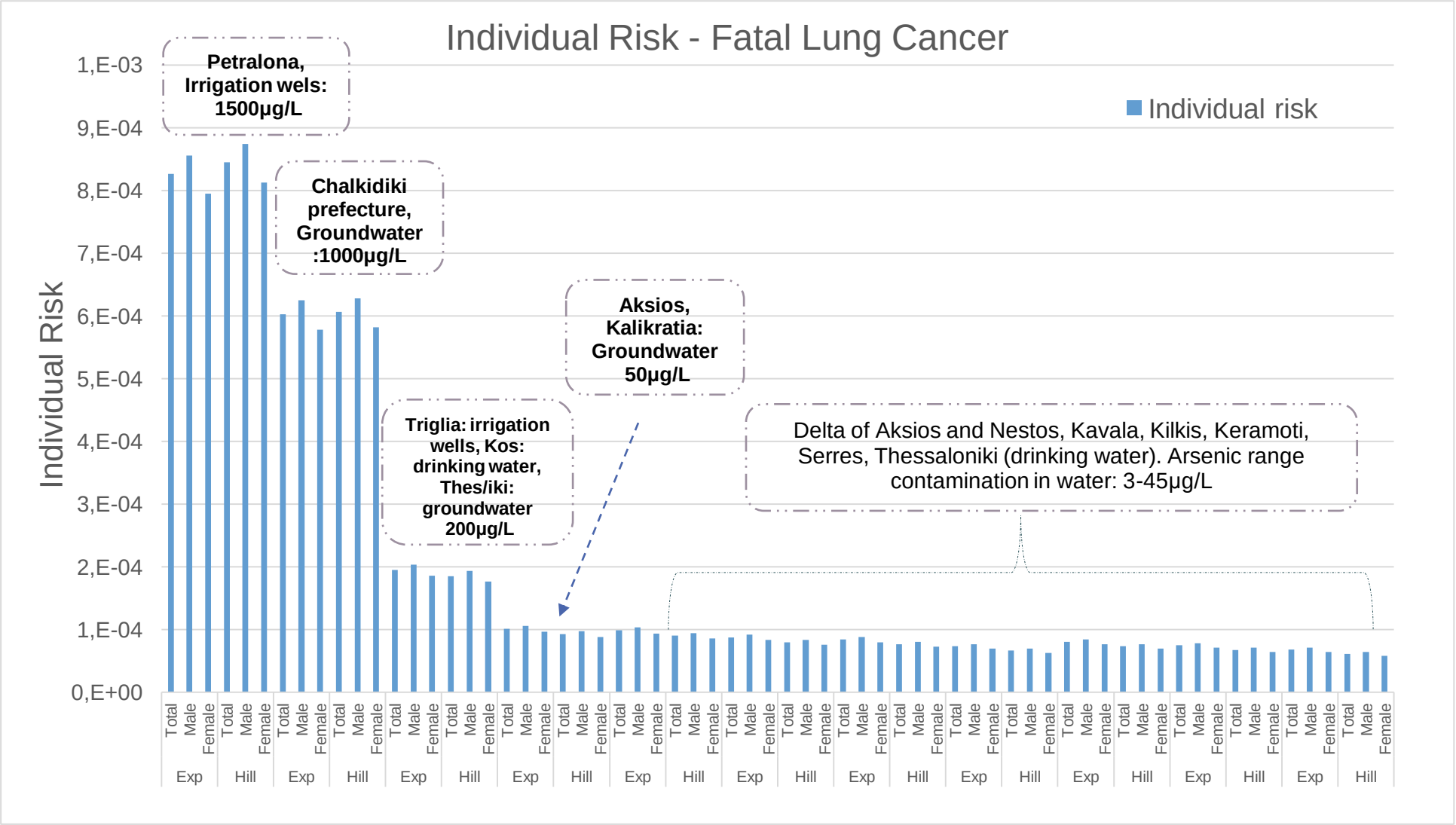

Figure 11. Individual Risk of Fatal Lung Cancer to several areas in Greece.

BIOMONITORING BASED EXPOSURE SCENARIO

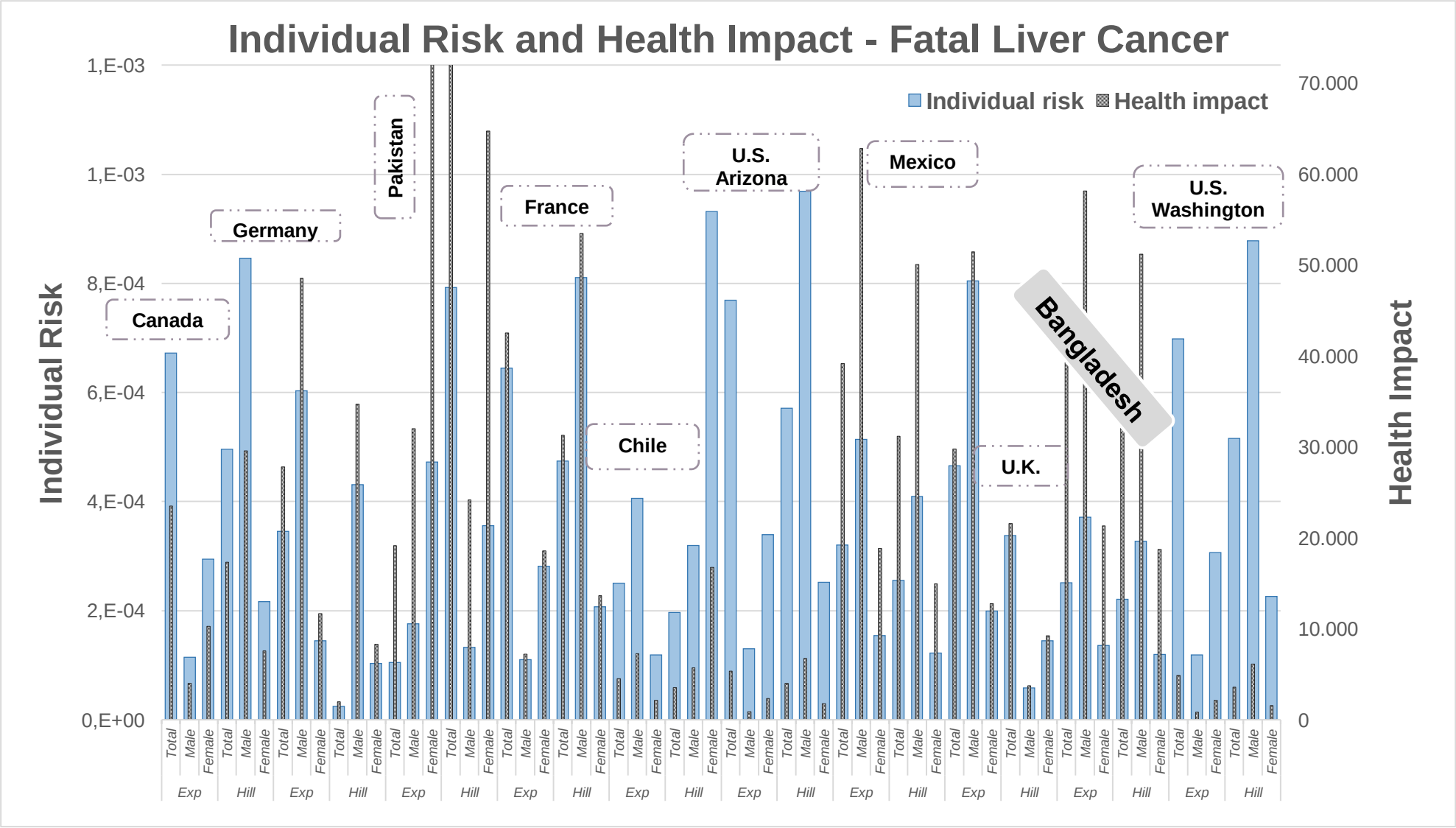

Figure 12.Individual Risk and Health Impact assessment of fatal liver cancer using biomonitoring data from different countries

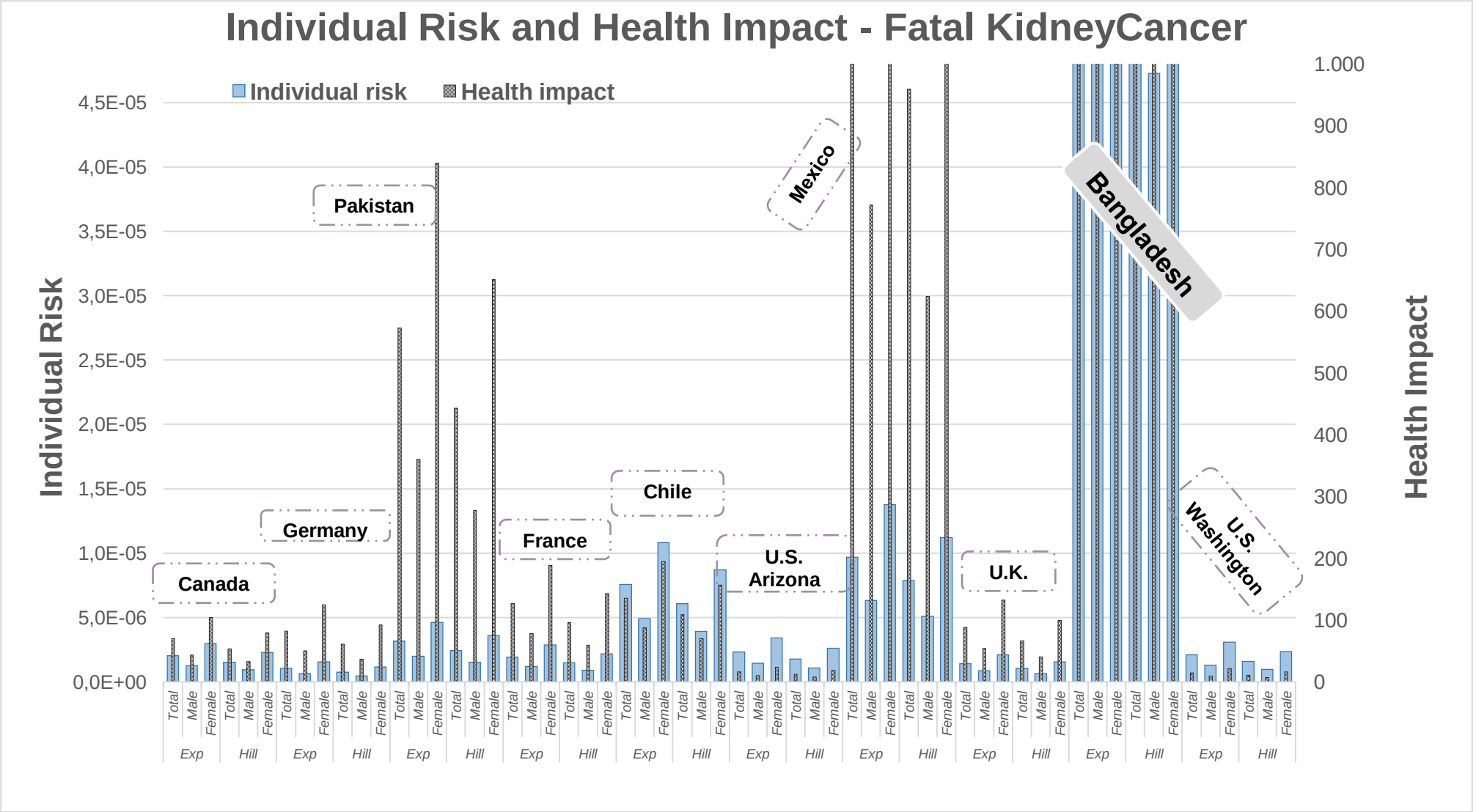

Figure 13. Individual Risk and Health Impact assessment of fatal kidney cancer using biomonitoring data from different countries

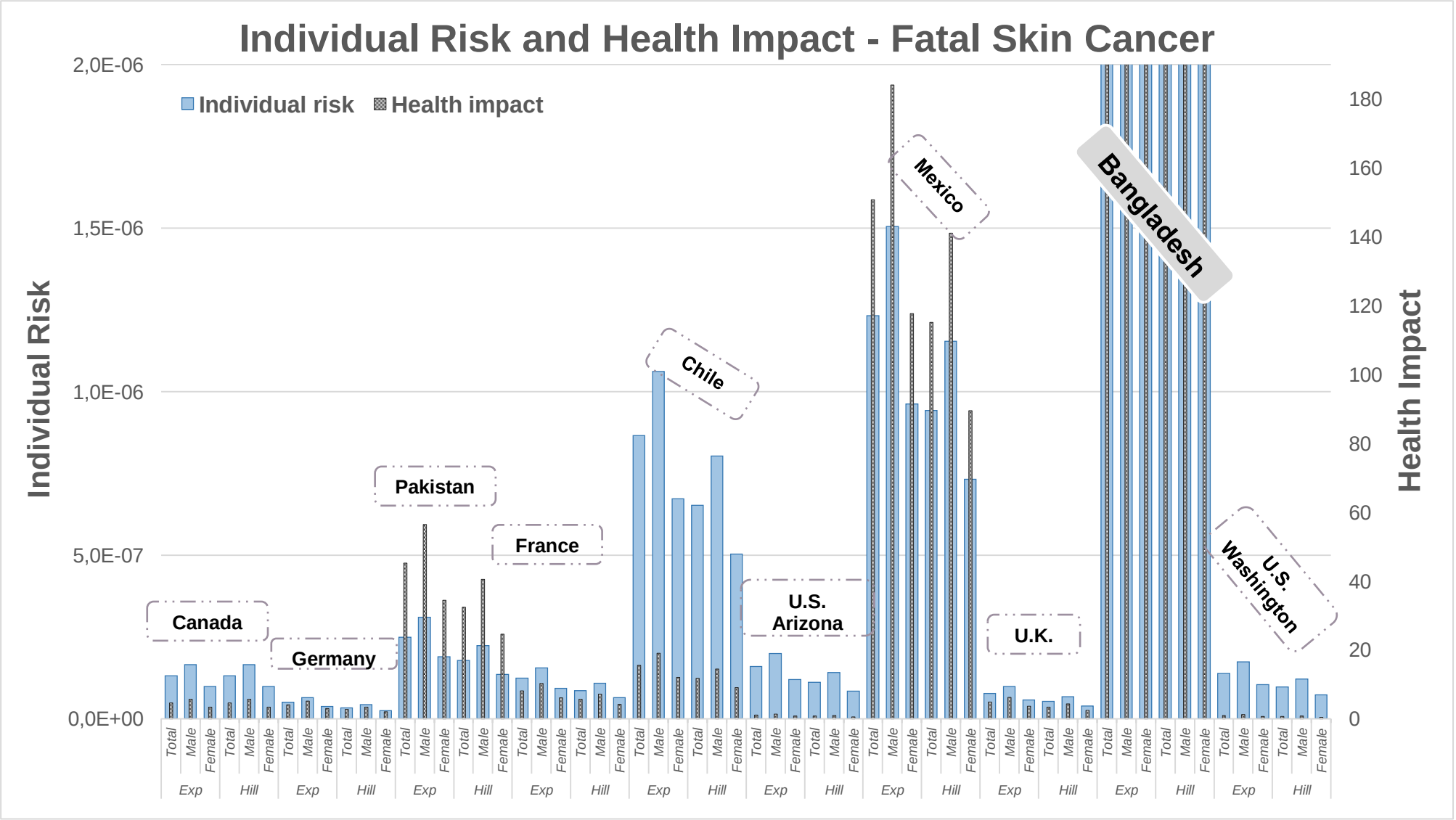

Figure 14. Individual Risk and Health Impact assessment of fatal skin cancer using biomonitoring data from different countries

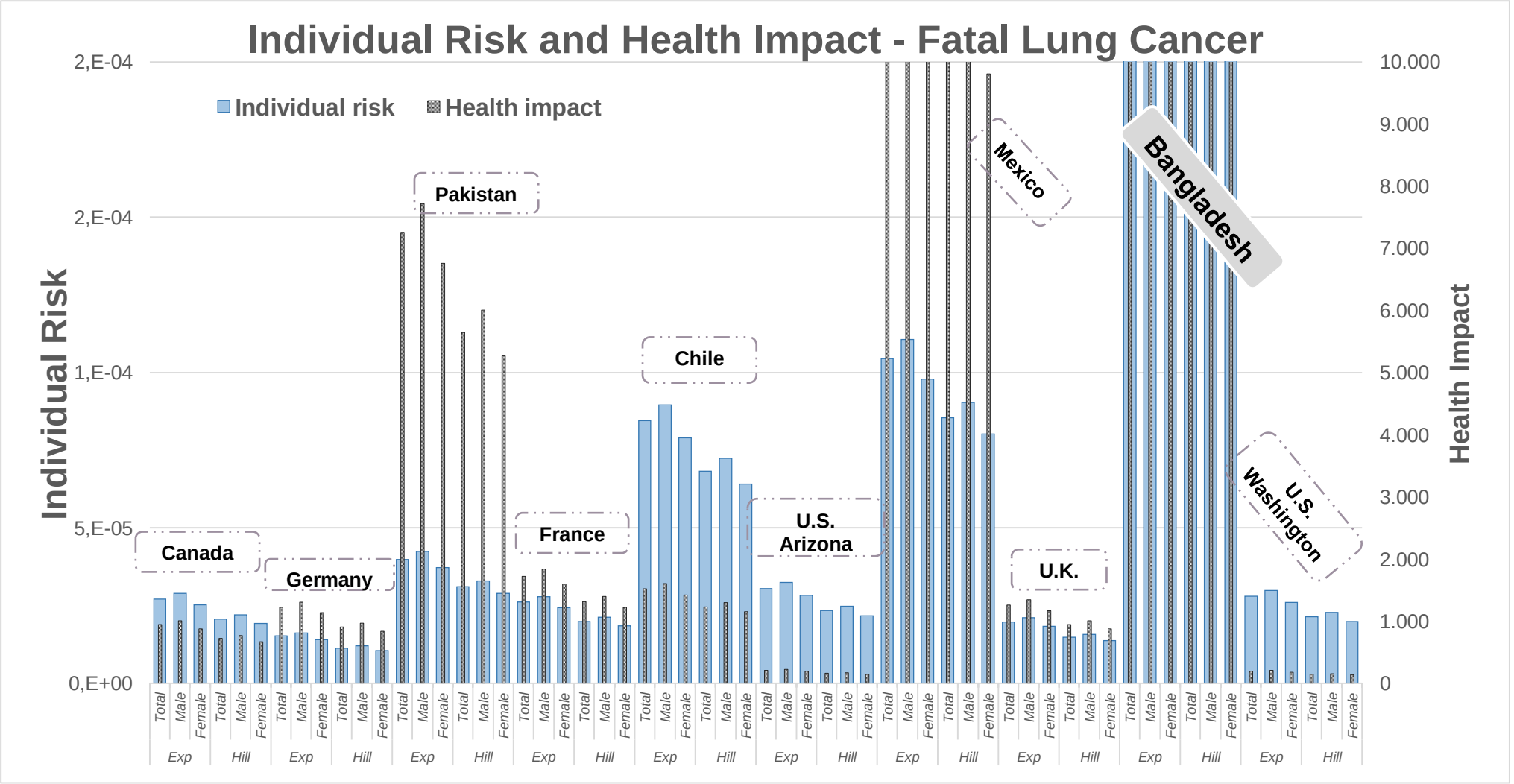

Figure 15. Individual Risk and Health Impact assessment of fatal lung cancer using biomonitoring data from different countries

DISCUSSION

Arsenic is an ubiquitous mineral in nature and its acute and chronic toxicity has been well documented. Arsenic contamination of groundwater is widespread and there are a number of regions where arsenic contamination of drinking water is significant. The goal of the present study was to estimate an overall risk of fatal cancer of Greek population due to external daily arsenic exposure from dietary and non-dietary pathways. In this case study we estimated the individual risk of exposure to arsenic and its metabolites via ingestion of food and water and inhalation, using both the developed PBPK model and the pathology model for the Greek population. Dose response functions based on two different statistical methods were used, namely the exponential formulation and the Hill equation. In both cases, the dose-response functions showed an approximately linear relationship between dose and health response at low doses.

A positive association between arsenic exposure and internal organ cancer (lung, liver, kidney, skin) has been indicated. The fact that DMA is produced by methylation in the liver, excreted via the kidneys and later stored in the bladder accounts for other tumor localizations. The fact that humans excrete more MMA than any other species may be a factor in their apparently higher sensitivity to arsenic-induced carcinogenesis. The evaluation of arsenic carcinogenicity can be summarized (IARC, 2004):

- There is sufficient evidence in humans that arsenic in drinking-water causes cancers of the bladder, lung and skin.
- There is sufficient evidence in experimental animals for the carcinogenicity of dimethylarsinic acid.
- There is limited evidence in experimental animals for the carcinogenicity of sodium arsenite, calcium arsenate and arsenic trioxide.
- There is inadequate evidence in experimental animals for the carcinogenicity of sodium arsenate and arsenic trisulfide.
- Taken together, the studies on inorganic arsenic provide limited evidence for carcinogenicity in experimental animals.

The exact mode of action of arsenic remains puzzled. More laboratory and clinical research is needed to define the mechanisms by which arsenic induces cancer to clarify the risks at lower doses. Skin, lung, liver and kidney fatal cancers have been chosen as health end-points, as the riskiest organs. In 2014, 11.321 cases of fatal cancer were reported in Greece (0.1% of the total population) (http://www.statistics.gr/el/statistics/-/publication/SPO12/) while in 2012 the 9.975 had been reported. From 11.321 deaths, 7.2% were malignant cancer of peritoneum organs, 6.6% cancers of respiratory system, 4.4%

neoplasms of urinary organs and 2.4% skin cancers. Those epidemiological reviews corroborate well with our estimates where lung fatal cancer was the most noteworthy highest individual risk for both males and females, followed by liver, kidney and skin.

Individuals ingest water both directly, and indirectly through ingestion of water added to food and drinks as part of preparation. Total drinking water intake refers to the ingestion of water for both drinking and food preparation purposes (Hough et al., 2010). In this study, water used for food preparation was not taken into account. In addition, water consumption rate may differ from winter to summer, especially in Greece where high temperatures remain unaltered during summer, expanding the need of consumption. A consumption rate of 2L/day was assumed in this study without considering the water added for food preparation and varieties in the amount of drinking water through the season. Arsenic residues were estimated in several food items and tap water, considering the contamination of those from groundwater. Starting from known arsenic levels groundwater in different areas in Greece and using the estimates of WATSON environmental fate model for assessing As multimedia environmental fate, we estimated the contamination of food items and tap water. In this study the differences of the daily intake for the age dependent dietary was not accounted. In addition, differences in the diet among the Greek residents were also not taken into account.

When groundwater contamination was considered, nourishment intake changed significantly; for instance, example in the scenario where groundwater is contaminated with 1000μg/L, the daily food intake dose was 8.5 mg/day, while in case where food contamination was not considered (e.g. 30μg/l in drinking water in Kavala), intake from food was estimated equal to 1mg/day. This highlights the importance of considering the complete environmental fate of arsenic including food chain. In this case, food is another major contributor to arsenic exposure as well. As a result, critical epidemiological reviews carried out in arsenic contaminated areas (e.g. Taiwan and Bangladesh) which were utilized to derive carcinogenic guidelines from world organizations, might lack the incomplete exposure picture due to inadequate exposure (including food intake) data. Thirteen different scenarios were evaluated for various regions in Greece where data were available. The arsenic contamination levels in groundwater ranged from 2.9 μg/L to 1000 μg/L. The total arsenic daily intake from tap water was 0.05-30.8 μg/kg bw (3.5-2157 μg/l or 0.03-21 mg/day). The assessments of total dietary arsenic intake were 1.02-12.41 μg/kg bw (71.4-854.7 μg/day or 0.7-8.5 mg/day). Lastly, the total arsenic daily intake was estimated: 1.08-32.3μg/kg bw (75.6-2262 μg/day or 0.76-22.6 mg/day). These estimates could be used to contrast the Greek population risk with regulatory thresholds.

Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) 2010 (WHO, 2010), determined the inorganic arsenic BMDL0.5 in human: 3 μg/kg b.w./day (ranged 2-7 μg/kg b.w./day). The $BMDL_{0.5}$ was identified for malignancies of the lung and skin, as well as skin lesions (Chain, 2009). The values of our interest is the range referring to total arsenic exposure estimates from our study: 1.08-32 μg/kg b.w/day. In case of total arsenic exposure, the value 1.08μg/kg b.w/day is based to exposure of 2.9 μg/L in groundwater resulting to 1 μg/kg b.w./day from food, and 0.05μg/kg. b.w./day from tap water, in areas such as Lesvos island, Kalloni Gulf. This is the minimum exposure scenario to inorganic arsenic considered in this work. The PBPK/BBDR model demonstrated an individual risk 10^{-6} developing fatal skin cancer, 10^{-5} for lung and liver cancer for both genders. 10^{-5} for kidney fatal cancer to females and 10^{-6} for males. The Benchmark Dose is the dose range within which arsenic is likely to cause a small but measurable effect on a human body organ. Even in the case of the minimum contamination (2.9μg/L) in groundwater, we are still in the scope of concerning risk (10^{-5} – 10^{-6}), thus, highlight the need of broader research concerning inorganic arsenic exposure to human health and eventual a revision of the standard values.

EPA has recommended a standard of 10 μg/L (10 ppb) for drinking water. In Serres, the drinking water arsenic concentration varies from 10 to 20 ppb. The simulation model reflected a similar response to fatal cancer between the two scenario with negligible differences. More in detail, in case of 10 and 20 ppb contaminated water the individual risk for skin, lung, liver and kidney range from 10^{-5} to 10^{-6} for both genders. Probability of fatal cancer to internal organs occurs also to concentration below 10ppm, which indicates the urgency of lowering the arsenic Maximum Contaminated Level.

Arsenic PTWI is 0.015 mg/kg b.w. Based to our results, total daily arsenic intake ranges from 1.08-32 μg/kg/bw. The PTWI is translated to a daily basis of 2.1 μg/kg b.w./day. The cases which PTWI is exceeded concern the following areas: Petralona, Chalkidiki prefecture, Triglia, Island of Kos, and Thessaloniki NW. The total arsenic daily intake in those areas were estimated 32, 12, 5.6, 3.29 μg/kg b.w. respectively (the other areas had values <2.1 μg/kg b.w./day). The contaminated groundwater in those areas is 1500,1000, 200 μg/L. respectively. The individual risk for the most organs ranges from 10^{-4} – 10^{-5} and 10^{-4} for lung cancer. In the other areas, where PTWI is below the standard range (1.08-1.61 μg/kg b.w./day) the individual risk generally ranges from 10^{-5} – 10^{-6} for kidney and skin, 10^{-5} for fatal lung and liver cancer. The PTWI is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects (WHO, 2007). The Minimal Risk Levels (MRLs) for acute oral exposure of arsenic is 5μg/kg

bw/day while for the chronic oral exposure the MRL is 0.3 μg/kg bw/day (ATSDR, 2016) where all the scenarios exceed the chronic oral exposure standard. The Committee noted that the established provisional tolerable weekly intake (PTW1) 2.1 μg/kg b.w./day for inorganic arsenic was in the region of the BDML05 and therefore was no longer appropriate (WHO, 2010). This PTW1 was therefore withdrawn by the Committee. No new tolerable intake level could be established.

Based on studies found in the literature (Ferreccio et al., 2000) it was found that after systemic exposure of 0.077mg/day of arsenic, lung cancer is developed to humans. Our results for arsenic exposure are 0.7 - 22 mg/day. Those estimated values, compared to toxicological studies in the literature, compared to the individual risk found from PBPK/BBDR model (10^{-4}), confirming the great risk some regions in Greece are. Other studies in humans are found in the literature to confirm the risk found in this case (Guo, 2004; Lubin et al., 2000; Welch et al., 1982; Zaldivar et al., 1981). Another Important finding to be noted is the fact that a safe level for arsenic in the air has not been established yet (WHO, 2010).

Several studies worldwide have been made to estimate the total arsenic exposure. A study in the U.S estimated the total inorganic arsenic intake from food, water, soil ingestion and from airborne particle inhalation. The results ranged from 1.8 to 11.4 μg/day for males and from 1.3 to 9.4 μg/day for females (Meacher et al., 2002). However, another study in U.S showed that exclusively dietary intake may range from 1 to 20 μg/day (Johnson, 2007). Those studies recommend that arsenic contamination usually varies within a country, implying that exploration of arsenic contamination in water must be done at individual residues of the same country. Arsenic exposure routes and chemistry for the general population are more complex, because it varies according to several factors, such us: geochemistry, local pollution, living conditions, lifestyles, and activity patterns of the exposed populations. Additionally, the 1/3 of the total population smokes cigarettes, highlighting the need of exposure studies to arsenic due to smoking.

Outlining our results, the individual risk in Greece for skin fatal cancer range from 10^{-4} in highly contaminated irrigation wells reaching the amount of 1500μg/L, in areas such as Petralona (Chalkidiki), to 10^{-6} in zones where the concentration in groundwater or drinking water shifts from 2.9 to 50μg/L. In instances of Chalkidiki prefecture, Triglia or Kos island, groundwater and drinking water separately reach the amount of 200μg/L, the individual risk is 10^{-5}. It has also to be noted that exposure to arsenic results in gender dependent differences in response. Males showed higher risk (one-fold range of magnitude up) contrasted with females. Males are more prone to develop skin cancer since ultraviolet exposure and stress induce immunosuppression in the human skin, and this effect is stronger in males (Dorak

and Karpuzoglu, 2012). Males additionally appear to be at more serious hazard compared to females to develop lethal liver cancer. There are known physiological distinction that may clarify this difference between gender (Dorak and Karpuzoglu, 2012; Naugler et al., 2007). For instance, in the worst scenario where groundwater is tainted with 200μg/L (Thessaloniki NW area) of arsenic, the individual risk for liver fatal cancer is one-fold range of magnitude up for males compared to females (10^{-4} versus 10^{-5}). The individual risk for fatal kidney cancer, range from 10^{-4} for females and 10^{-5} for males up to 10^{-6}. Females presented a higher individual risk compared to males (one-fold order of magnitude). This might be explained from gender dependent differences in toxicokinetics, since women present a higher capacity from methylation of MMA (Hsueh et al., 1998). MMA is methylated to DMA^V which is more toxic to the female urinary bladder, which is reflected as sensitivity to carcinogenesis (Shen et al., 2006). The urinary system includes also the kidneys; we can assume that there may be a link of higher toxicity of DMA^V to female kidneys. Additionally, the methylation of inorganic arsenic may be a toxificaton-activation process, due to the great biological activity of trivalent methylated arsenic metabolites with proteins and even DNA (Kitchin, 2001). Males have higher morbidity and mortality rates from lung cancer due to higher frequency of smoking, but female are at greater risk to develop it (Kiyohara and Ohno, 2010). These sex disparities were relatively modest. The individual risk for lung cancer is the highest in concentration of 200μg/L arsenic in water (10^{-4}) compared to the other organs. In our study, males demonstrate higher risk than the females in most of the cases (lung, liver and skin), while females show an increased risk on fatal kidney cancer. Different physiological and bioaccumulation properties on women and men may influence distribution of chemical as well as the extent rate of accumulation and release from adipose tissue. The gender difference in cancer susceptibility is one of the most consistent findings in cancer epidemiology (Dorak and Karpuzoglu, 2012). In general, males have worse overall survival, higher mortality rates due to cancer (44.85% for males, instead of 38.08% for females) and a lifetime probability of developing cancer (Cook et al., 2011; Greenlee et al., 2000). Universal mechanisms related to gender differences in cancer incidence and, thus, mortality include antioxidative capacity, gender chromosome complement, aneuploidy, aberrations, gene expression, hormones, and immunocompetence. Biological factors may influence kinetics and toxicity of chemicals, which behave differently in men and women, sometimes under the direct influence of sex hormones (Vahter et al., 2007). Other gender differences include lifestyle factors such as: exposure conditions in the working or general environment, smoking, dietary factors, physical activity, cosmetics use and stress factors. The different associations between arsenic and various cancers deserve further exploration including the gender.

Assessment from biomonitoring urinary excretion data exhibited a lower individual risk, in both genders. Although biomonitoring provides the most complete picture of intake from multiple pathways and sources, uncertainties related to this process have to be discussed. This are mainly attributed to the rapid arsenic excretion via the urinary system, hence, the use of spot samples might result in intake underestimates. In addition, several factors play an essential role, such as the frequency of sampling, the creatinine clearance, as well as gender, age and genetic factors that affect metabolism and renal excretion susceptibility. In addition, the inconsistency of the analytical methods results in different limits of detection, sensitivity, accuracy, introducing additional uncertainty in the results. It is noteworthy that exchange among the various forms of reduced and oxidized arsenic forms can occur also non-enzymatically, depending on oxygen tension, pH and presence of endogenous reductants, making the measurement of arsenic in biological samples difficult (Thomas et al., 2004). The total arsenic intake (μg/kg bw) calculated from the biomonitoring data showed a broad range between the countries. The highest intake estimates (14 μg/kg bw/day) were calculated for Bangladesh, resulting in the highest individual risk in men and females (10^{-5} - 10^{-6}), as well as in the highest mortality rates (868-3454). Arsenic in Bangladesh has attracted much attention since 1990s, as a result of the high contamination levels in well-water. Since this time, significant progress has been made and the number of people exposed to arsenic levels exceeding the Bangladesh drinking-water quality standard has decreased approximately 40%. Despite these efforts, it has been estimated that about 20 million to 45 million people in Bangladesh are at risk of exposure to arsenic levels higher than the national standard of 50 μg/L and the WHO guideline level of 10 μg/L respectively. Today, arsenic compounds are regulated in Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the REACH. In Annex XVII, amended in 2009 (ECHA, 2009), it is stated that arsenic compounds shall not be placed on the market, or used, as substances or in mixtures, intended for use to prevent the fouling by micro-organisms, plants or animals. On the other hand, the lowest intake of 0.15 μg/ kg bw was estimated in Germany, where the individual risks ranged from 10^{-6} in the case of lung cancer to 10^{-5} for fatal skin cancer, and a respective low health impact of 0.3 for skin mortality to 90 cases for lung mortality.

The model makes the following significant assumptions: All chemical transport is based on passive and facilitated transport mechanisms; dermal uptake is insignificant and is not included; the chemical is lost only by reaction, urination and exhalation; the modeled human is an adult 70kg, and is not growing; the physiological processes and parameters do not change as a result of prolonged chemical exposure. Systemic circulation of MMA^{III} and DMA^{III}, which are formed by the reduction of the pentavalent form, in not considered. Exposure to organic arsenical forms (DMA, MMA) to the initial dose are not considered. To determine the biologically relevant target tissue dose and health effects we must consider the presence of other contaminants (Diacomanolis et al., 2014; Hays et al., 2006) and the Individual variability in metabolism, depending on genetic makeup and developmental stage (Meza et al., 2005; Skroder Loveborn et al., 2016) Factors such as dose, age, gender (Hsueh et al., 1998; Shen et al., 2006), ethnicity, (Meza et al., 2005) and smoking contribute only minimally to the large inter-individual variation in arsenic metabolism observed in humans due to genetic polymorphisms (Yu et al., 2003), nutrition and dose exposure. Future studies should be designed incorporating the susceptibility to arsenic (e.g., smoking, diet, genetics). Gender-related differences in methylation have been reported, which results in women indicating a higher capacity for methylation of monomethylarsonic acid (MMA) (Hsueh et al., 1998). DMA^{V} is more toxic to the female urinary bladder, in accord with sensitivity to carcinogenesis (Shen et al., 2006).

Although the internal dosimetry modelling provided major advantages for assessing the risks of arsenic, there are additional challenges that have to be addressed, so as to further advancing the description of toxicokinetics. Future work may involve a thorough assessment on differences of chemical impact on human body in relation to gender. Different physiological and bioaccumulation properties on women and men may influence distribution of chemical as well as the extent rate of accumulation and release from adipose tissue. Vahter et al. (2007) reported, for example, gender differences in the vulnerability of the kidney to damage or cancer subject to a toxicant, and more generally, differences in rates of cancer in organ such as brain, kidney, liver, GI tract, and dermal system. The studies of gender differences should involve both PBPK modelling and dose response modelling. Similarly, age dependent differences should be taken into account. This however would require an extensive data set, which, especially for PBPK modelling, is missing. Information on physiology and biology in different ethnic populations and disease group are also lacking. PBPK modeling is considered to be complex and data intensive. However, as our knowledge of physiology and biochemical processes improves, especially in different disease states, even more sophisticated models will be developed. PBPK models are a very promising tool (Huang et al., 2013; Rostami-Hodjegan et al., 2012) that will provide additional insides in the

risk assessment process. The use of PBPK modelling in understanding exposure and improving risk assessment and health impact is widely recognized, and PBPK models are incorporated in computational platforms for exposure and risk assessment (Sarigiannis et al., 2015) (Georgopoulos et al., 2008).

DECONTAMINATION OF DRINKING WATER

Greece seems to be highly contaminated in some areas such as the Chalkidiki prefecture which is a highly visited and touristic area in summer. The most important action in affected communities is the prevention of further exposure to arsenic by the provision of water with minimum As content for drinking, food preparation and irrigation of food crops. There are a number of options to reduce levels of arsenic in drinking-water.

PHYSICOCHEMICAL TECHNOLOGIES

Lime precipitation: Lime precipitation has been used to reduce arsenic concentrations from high levels (e.g., hundreds of mg/L) to moderate levels (e.g., 1 to 5 mg/L). Precipitation is typically followed by clarification or filtration for solids removal.

Oxidation: Oxidation is a chemical process typically used in conjunction with other processes for arsenic removal. As3+ (As(III) or arsenite) is more soluble in water and less available for precipitation/adsorption reactions than its As5+ (As(V) or arsenate) relative.

Coagulation/filtration: A cost-effective approach for arsenic removal is coagulation and precipitation (chemical processes) followed by filtration (a physical process), which is termed coagulation/filtration (CF) in the water treatment industry. Common coagulants used for arsenic are iron salts and aluminum sulfate (alum).

Adsorptive media (AM): AM is another common technology for arsenic removal that can be used in place of or to augment CF. As with coagulants, most adsorptive media are iron-based; variations include titanium dioxide, zirconium, and other ion exchange resins.

Ion Exchange (IX): The ion exchange (IX) process differs from the AM process in that IX media is meant to be regenerated periodically and reused after arsenic adsorption. IX media is typically regenerated with sodium hydroxide and sodium chloride, which creates a liquid waste containing a high concentration of arsenic. Since waste disposal may be problematic, IX is not typically used for arsenic removal.

Reverse osmosis: Membrane separation technologies are attractive arsenic treatment processes for small water systems. RO is a pressure-driven membrane separation process capable of removing arsenic from water by means of particle size, dielectric characteristics, and hydrophilicity/hydrophobicity.

BIOLOGICAL METHODS

It was shown that arsenic can be removed by direct adsorption or co-precipitation on the preformed biogenic iron or manganese oxides, whereas the oxidation of As(III) was induced by the iron-oxidizing bacteria and leads to improved overall removal efficiency of arsenic content.

Biological manganese removal: The removal of dissolved manganese (Mn2+) from groundwaters is generally accomplished by oxidation, followed by precipitation and (sand) filtration for the removal of the oxidized insoluble products (Knocke et al., 1991). Iron and manganese are often associated with elevated arsenic concentrations of geogenic origin in groundwaters (Katsoyiannis et al., 2007a; Rowland et al., 2011). The distribution of inorganic arsenic species [As(III), As(V)] in natural waters is mainly dependent on redox potential and pH conditions. Generally, adsorption of As(III) onto iron or manganese oxides is less efficient than of As(V); therefore, the immobilization of As(III) is enhanced by the preliminary oxidation of As(III) to As(V) (Katsoyiannis and Zouboulis, 2006).

Biological iron removal: Iron-containing groundwaters have been traditionally treated by chemical oxidation, promoted with the vigorous aeration and/or the addition of chemical oxidizing agents

Use of plug flow reactors combined with microfiltration: A modification of the traditional biological iron and manganese oxidation taking place in fixed bed bioreactors is the use of plug flow reactors followed by membrane microfiltration (Katsoyiannis et al., 2013). The PR-MF (hybrid plug flow reactor-microfiltration (PR-MF)) process efficiently removed iron, manganese, and arsenic without the use of chemical reagents for oxidation or pH adjustment, and without the need for regular regeneration or backwashing, following the principles of green chemistry.

Phytoremediation for arsenic removal by aquatic macrophytes: Phytoremediation of toxic contaminants can be readily achieved by aquatic macrophytes or by other floating plants since the process involves biosorption and bioaccumulation of the soluble and bioavailable contaminants from water. A large number of aquatic macrophytes have been studied for the phytoremediation of toxic metals from waters, such as Microspora and Lemna minor and Typha latifolia (Chakrabarty, 2015).

Arsenic removal by bacteria and algae: In water treatment, the critical step is the oxidation of As(III) to As(V), because As(V) is more efficiently removed by traditional methods, such as coagulation with iron and aluminum salts, ion exchange, lime softening, and adsorption on specific media. Therefore, in water treatment, the identification of bacteria that can oxidize

As(III) is of high importance. The main bacteria that are able to oxidize As(III) are iron-oxidizing bacteria, such as L. ochracea or G. ferruginea, which work well at pH values relevant to groundwater treatment and therefore have found wide application in water treatment plants, as described earlier in the text (Chakrabarty, 2015).

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