Improved Integrated Risk Assessment of Geogenic Arsenic: Exposure and Attributable Health Risks

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 2

H. Rasheed, R. Slack, P. Kay. 2016. Human health risk assessment for arsenic: a critical review. Critical Reviews in Environmental Science and Technology. Volume 46, 2016 - Issue 19-20.

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HR (the candidate) collected the literature, conducted all of the data analysis, and made the figures and wrote the manuscript. RS and PK provided advice and guidance throughout and gave feedback on several drafts of the manuscript.

Chapter 4

Rasheed H; Slack R; Kay P; Gong YY. 2017. Refinement of arsenic attributable health risks in rural Pakistan using population specific dietary intake values, *Environment International*, 99**,** pp.331-342.

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HR developed the questionnaires, conducted field visits including interviews, applied for ethical review, conducted the data analysis, prepared the figures and wrote the manuscript. RS helped in obtaining the ethical review. RS and PK contributed with critical feedback on field questionnaire and several drafts of the manuscript. YG reviewed the final draft of the manuscript.

Chapter 5

Rasheed H; Kay P**;** Slack R; Gong YY; Carter A. 2016. Human exposure assessment of different arsenic species in household water sources in a high risk arsenic area, *Science of the Total Environment*.

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HR developed the sampling strategy, prepared the instruments for fieldwork, collected samples, acquired the laboratory facilitation for speciation analysis, performed the data analysis, made all the figures and wrote the manuscript. AC prepared the samples for chemical analysis. PK, RS and YG provided guidance on data interpretation and provided critical feedback on two drafts of the manuscript.

Chapter 6

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HR developed the sampling strategy, prepared the instruments for fieldwork, collected samples, partly analysed the samples, acquired the laboratory facilitation for speciation analysis, performed the data analysis, and prepared all the figures and wrote the manuscript. All co-authors provided guidance on data interpretation and manuscript draft.

Chapter 7

Rasheed H; Kay P; Slack R; Gong YY. Assessment of arsenic species in human hair, toenail and urine and their association with water and staple food (forthcoming in Journal of the Exposure Science and Environmental Epidemiology).

HR applied for ethical review, conducted fieldwork, acquired the laboratory facilitation for speciation analysis, performed the data analysis, and prepared all the figures presented in the manuscript and wrote the manuscript. RS helped in obtaining ethical review and acquiring the laboratory facilitation for speciation analysis. YG helped in guide the analysis and in the output interpretations. PK gave critical feedback on a manuscript draft.

Chapter 8

Rasheed H; Kay P; Slack R; Gong YY. 2017. The effect of association between inefficient arsenic methylation capacity and demographic characteristics on the risk of skin lesions. *Toxicology and Applied Pharmacology.* https://doi.org/10.1016/j.taap.2017.11.026**.** *(Published).*

HR applied for ethical review, conducted fieldwork including interviews and physical examinations, acquired the laboratory facilitation for speciation analysis, analysed data, prepared all the figures and wrote the manuscript. RS helped in obtaining ethical review and acquiring the laboratory facilitation for speciation analysis. YG guided in the data analysis and PK gave critical feedback on manuscript drafts.

Chapter 9

Rasheed H; Kay P**;** Slack R; Gong YY. Integrated health risk assessment for arsenic: multiple exposure sources and arsenic species. *(In Review in Environmental Health Perspective).*

HR conducted the health risk modelling and data analysis, and prepared the figures and wrote the manuscript. PK, RS and YG contributed with critical feedback on a draft of the manuscript.

Thesis by Alternative Format Rationale

This thesis is submitted in accordance with the Faculty of Environment's alternative style of doctoral thesis including published material. This format is apposite for this thesis because four out of the seven data chapters has already been published in peer-reviewed journal, three are currently in review. Loose copies of the published manuscript accompany this thesis. The six manuscripts based on current research findings are preceded by an introduction (Chapter 1), including an outline of the main research questions and the novelty of the research, a critical literature review (Chapter 2), general methodology (Chapter 3), research synthesis and conclusions (Chapter 10) follows the research articles, and intertwines the findings of all six manuscripts, placing them in the context of the objectives and the literature, providing critical discussion and including guidelines for future work. This is in line with the alternative style of doctoral thesis including published material.

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Abstract

The linkage between arsenic contaminated water and increased cancer risk is well recognized. The potential health risk posed by separate inorganic and organic arsenic species through combined exposure to arsenic contaminated water and staple foods is not well understood though. Therefore, this research aims to improve arsenic risk assessment by investigating the primary exposure sources, pathways, metabolism and response indicators in an integrated manner. The population based water and food consumption pattern characterised by this research was used to validate the cancer risk modelling which demonstrated that using water or food intake values from the developed world may not represent cancer risks to the specific population in question. Integrating this characterisation with arsenic species provided several key insights. Arsenate was identified as the main species in the ground water aquifers of five villages whilst the predominance of arsenite and its co-existence with arsenate in one village indicated variations in aquifer redox conditions. Wheat cultivated with arsenic-rich irrigation water proved to be an alternate exposure pathway of inorganic arsenic. The species specific probabilistic cancer and non-cancer risks were found to be higher for arsenite followed by arsenate, whilst no risk was found for dimethylarsinic acid of dietary origin. The comparative impact of various reference doses on chronic health risk substantiated that children are at higher vulnerability, whilst using population based exposure characteristics of this study population and relative risk estimates from southwest Taiwan, showed females to be at higher risk of life time bladder and lung cancer due to inorganic arsenic. No risk was associated with low doses of arsenic. Total ingested arsenic from water or food under the effect of certain potential modifiers was a significant predictor of arsenic species in human biomarkers and proved toenail to be a comparatively effective biomarker. At low arsenic levels in water, food associated total arsenic was a better predictor of urinary metabolites. The total arsenic intake from water and urinary metabolites under the effect of labour jobs strongly predicted the increased risk of arsenical skin lesions. Probabilistic risk modelling indicated that persons with skin lesions were at higher risk of transformation of skin lesions into skin cancer, also evidenced with their lower methylation capability.

Overall, this thesis provides evidence that species based risk assessment requires a greater understanding of exposure matrix, toxicological thresholds and metabolic reactions from ingestion to potential endpoints. This study has provided a baseline of inorganic arsenic for risk management to set public health water supply goals and to minimize the daily consumption of cooked rice for compliance with the safe arsenic limit. The findings are suitable to support future regulatory processes for species based arsenic limits in water together with staple foods.

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List of Abbreviations

ADAFs	Age-Dependent Adjustment Factors
ADD	Average Daily Dose
AF	Assessment Factor
AIC	Akaike Information Criterion
AM	Arithmetic Mean
ANOVA	Analysis of variance
As	Arsenic
AS3MT	Arsenic (+3 Oxidation State) Methyltransferase
AsB	Arsenobetaine
AsC	Arsenocholine
AsIII	Arsenite (Arsenous Acid)
AsV	Arsenate (Arsenic Acid)
ATe	Average life expectancy
ATSDR	Agency for Toxic Substances and Disease Registry
b	dose coefficient
BAL	Brooks Applied Laboratory
BBDR	Biologically-Based Dose–Response
BDL	Below Detection Limit
BEIR	Biological Effects of Ionizing Radiation
BFD	Blackfoot Disease
BHU	Basic health unit
BW	Body weight
C	Concentration
°C	Degree celsius
CAI	Cumulative Arsenic Exposure Index
CAL	Calibration Standard
CCA	Current Codex Alimentarius
CI	Confidence Interval
CR	Cancer Risk
CRC ICP-MS	Inductively Coupled Plasma Collision Reaction Cell Mass Spectrometry
CSF	Cancer slope factor
DALYs	Death and Disability Adjusted Life Years

DIW	Deionised Water
DMA or DMAV	Dimethylarsinic Acid
DMAIII	Dimethylarsinous Acid
DRC™	Dynamic Reaction Cell
DUP	Duplicate
EC	European Commission
ED	Exposure Duration
EDTA	Ethylenediaminetetraacetic Acid
EF	Exposure Frequency
EFSA	European Food Safety Agency
EU	European Union
FFQ	Food Frequency Questionnaire
F_{iAs}	Relative source contribution to iAs exposure due to drinking water
g day^{-1}	Grams per day
GEE	Generalized Estimating Equation
GFAAS	Graphite Furnace Atomic Absorption Spectrometery
GM	Geometric Mean
GP	Generalized Pareto
GPS	Geographical Positioning System
H_2O_2	Hydrogen Peroxide
HDPE	High Density Polyethylene
HEALS	Health Effects of Arsenic Longitudinal Study
Hgb A1C	Glycosylated Haemoglobin
HI	Hazard Index
HPLC	High-Performance Liquid Chromatography
HQ	Hazard Quotient
IARC	International Agency for Research on Cancer
iAs	Inorganic Arsenic
ICP-DRC-MS	Inductively Coupled-Plasma Dynamic Reaction Cell-Mass Spectrometry
ICP-MS	Inductively Coupled Plasma – Mass Spectrometer
ICV	Initial Calibration Verification
IR	Ingestion/Intake rate
JECFA	Joint FAO/WHO Expert Committee on Food Additives

kg day^{-1}	Kilogram per day
L day^{-1}	Litre per day
LB	Lower bound
LED_{01}	Lowest Effective Dose
LOD	Limit of Detection
MCLG	Maximum Contaminant Level Goal
MDLs	Method Detection Limits
mg day^{-1}	Milligrams per day
mg kg^{-1}	Milligram per kilogram
mg L^{-1}	Milligram per Litre
MIR	Mortality to Incidence Ratio
MLE	Maximum Likelihood Estimate
MMA or MMAV	Monomethylarsonic Acid
MMAIII	Monomethylarsonous Acid or Methylarsonous Acid
MOA	Mutagenic Mode of Action
MRLs	Minimal Risk Levels
MS	Matrix Spike
MΩ	Milliohm
NCS DC	China National analysis Centre for iron and steel, Beijing, China
NIST	National Institute of Standards and Technology
OR	Odd ratio
%Rec	Percent recovery
PBTK/TD	Physiologically Based Toxicokinetic and Toxicodynamic Model
PEMT	Phosphatidylethanolamine Methyltransferase Pathway
PHG	Public Health Goal
PMI	Primary Methylation Index
PRA	Probabilistic Risk Assessment
PTDI	Provisional Tolerable Daily Intake
PWTI	Provisional Weekly Tolerable Intake
QC	Quality Control
RfD	Reference Dose
RPD	Relative Percent Difference
RR	Raw rice

RYK	Rahim Yar Khan
SD	Standard Deviation
SMI	Secondary Methylation Index
SMR	Standardized Mortality Ratio
SRMs	Standard Reference Materials
SRRE	Summary Relative Risk Estimate
SSD	Species Sensitivity Distribution
SumAs	Sum of arsenic and its species
tAs	Total Arsenic
TDI	Total Daily Intake
µg g^{-1}	Microgram per gram
µg kg^{-1}	Microgram per kilogram
µg L^{-1}	Microgram per Liter
UB	Upper bound
UBC	Upper Baseline Concentration
UCL	Upper Confidence Limit
UCLs	Upper Confidence Limits
U-Cre	Urinary Creatinine
UNICEF	United Nations Childrens' Fund
UPS	Uninterruptible Power Supply
USEPA	Us Environmental Protection Agency
WHO	World Health Organization
YLDs	Years Lived with Disability

Chapter 1: General Introduction

1.1 Project rationale

Arsenic (As) is a naturally occurring metalloid that is widely distributed in the Earth's crust and exists in various chemical and biological forms. The four oxidation states of -3, 0, +3 and +5 reflect the capability of this element to adapt to any environment. Several epidemiological studies have proved arsenic to be a serious environmental and public health toxicant even at low concentrations in the human body (Hughes et al., 2011). The ingestion of arsenic was considered as the primary exposure route which results in various cancer and non-cancer health effects of uncertain etiology (National Research Council, 2013). The estimated population exposed to unsafe arsenic levels in groundwater in South and South-east Asia have been reported to be over 100 million (Ravenscroft et al., 2009). Arsenic enters the food chain mainly through this contaminated groundwater and, to a lesser extent, through agricultural pesticide and fertilizer applications, poultry feed supplements, release into soil and water through mining and smelting activities (Garelick et al., 2008). Nevertheless, variability in food sources and arsenic contaminated water used for irrigation as well as food preparation makes it challenging to differentiate the relative contribution of food in arsenic contamination.

Arsenic in rice is a current global issue as rice is a staple food for almost half of the world's population of 7 billion people and is of particular concern due to a 10-fold higher arsenic bioaccumulation rate in rice compared to other grains (Mohanty, 2013; Williams et al., 2007; Ma et al., 2008). In several countries people use both rice and wheat as staples, however the role of arsenic species in human disease development is still to be well understood. In addition to these staples, other dietary exposure sources and their consumption frequency have not been adequately prioritized and regulated, consequently, the preliminary advisory levels of 200 µg kg^{-1} inorganic arsenic (iAs) in polished rice grains set by Codex Alimentarius Commission (2014) is still debated.

The toxicity of arsenic is dependent on its chemical forms, its oxidation state and metabolic pathways of arsenic species within the human body (Irvin and Irgolic, 1995). Previous risk assessment studies (Chen et al., 2010; Saipan and Ruangwises, 2009; Meharg et al., 2009; Chen and Wang, 1990; Wu et al., 1989) were based on iAs exposure and have insufficiently taken into account different arsenic species due to laboratory analysis challenges. A risk assessment not considering arsenic species but assuming the presence of total arsenic (tAs) of dietary origin as iAs would lead to an overestimated health risk (European Food Safety Agency, 2009). Since the combined contribution of water and food and the influence of arsenic species on health risk have been inadequately assessed, the carcinogenicity of iAs emphasizes the need for inclusion of toxicologically important arsenic species (Figure 1.1) in risk assessment. The International Agency for Research on Cancer has categorized arsenic and arsenic compounds as carcinogenic to humans (Group 1) (International Agency for Research on Cancer, 2004)**.** On the basis of adequate evidence of cancer in animals (Arnold et al., 2006; Wei et al., 2002), MMA and DMA were grouped as possibly carcinogenic to humans (Group 2B) whilst arsenobetaine and other organic compounds were considered not classifiable for their carcinogenicity (Group 3)*.*

Arsenic induced cancer and non-cancer effects depend on the efficiency with which As is metabolized, accumulated and eliminated from the human body which in turn, depend on the dietary intake of As and its species. Furthermore, the difference of arsenic toxicity from dietary intake versus internal dose produced from metabolism is unclear due to the formation of highly reactive and genotoxic intermediate metabolites such as MMAIII (Cohen et al., 2006). Despite many studies on animals and humans (Yamamoto et al., 1995; Wanibuchi et al., 1996; Hughes, 2006; Chen et al., 2005), As metabolism and the potential risks posed by separate arsenic species of dietary origin under the influence of certain potential modifiers is only partially understood.

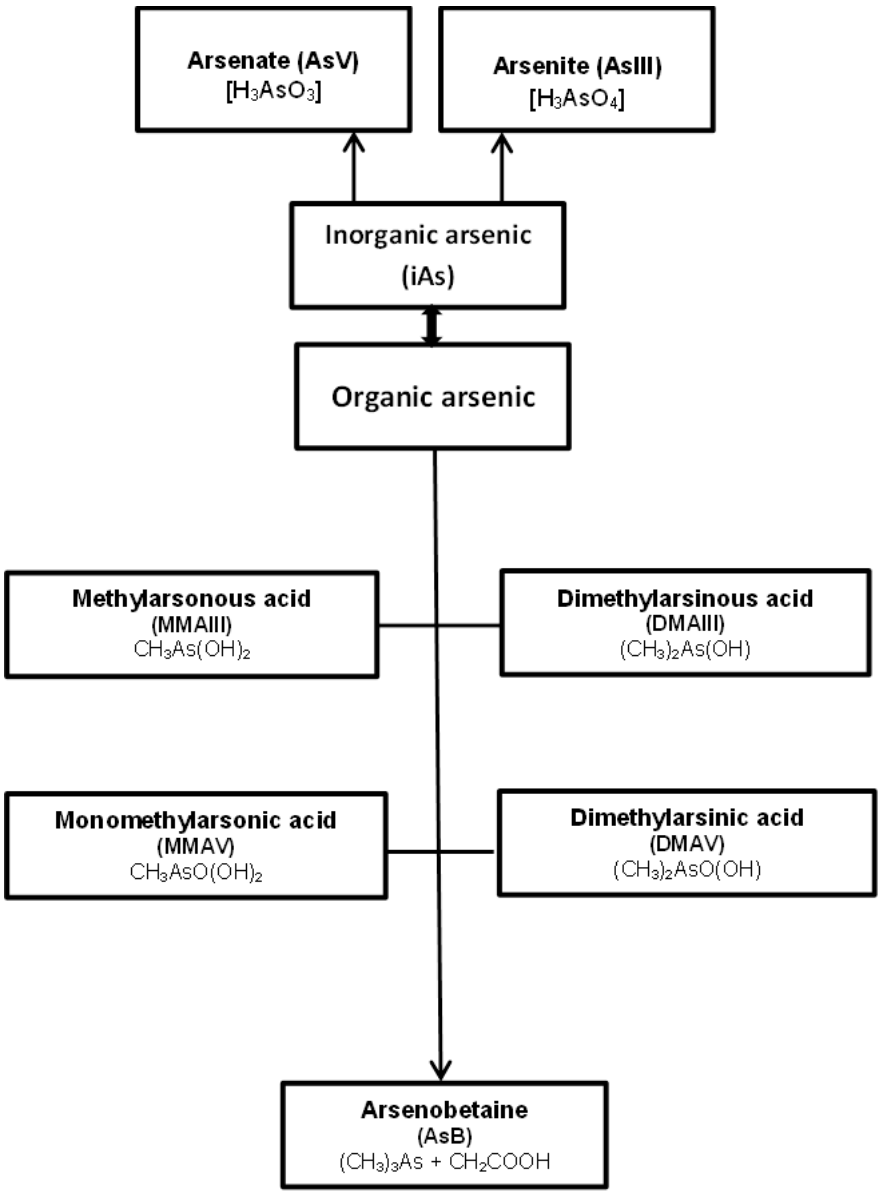

Figure 1.1 Inorganic and organic arsenic compounds and species typically found in water, soil and food

To expand the understanding of the impact of dietary exposure on As metabolism, accumulation and elimination of arsenic metabolites in individuals within a population will be helpful. In this context, population specific exposure characteristics defined by adopting a spatially intensive approach have significance for the realistic estimation of age and gender specific aggregate exposure and related health risk. Many past studies have quantified risk using generic or default exposure factors (e.g. average body weights, exposure duration, daily water or food intake, average life expectancy) set by US Environmental Protection Agency (2011) or World Health Organization (2011)

on the basis of studies conducted on developed world populations. These values when applied to the population of a different geographical region e.g. Izmir, Turkey (Kavcar et al., 2009), Vietnam (Nguyen et al., 2009), Pakistan (Muhammad et al., 2010) might have misrepresented the As dose that each individual in a population was exposed to through multiple sources. Consequently the substantial difference in As metabolism and resulting health impacts at a range of arsenic concentrations would not be demonstrative of the country or population in question.

Low dose health risk is still controversial due to conflicting evidence provided by dozens of studies as reported by Schmidt (2014), however the recognition of arsenic as a human carcinogen by the International Agency for Research on Cancer (2012b) provides a strong basis to raise concern even at lower exposure level. These gaps in the area of human health risk assessment provided a strong argument for the need to refine arsenic risk assessment by adopting an integrated risk assessment approach based on the primary hypothesis that dietary intake of trivalent and methylated arsenic species make a significant contribution to potential health risks.

1.2 Aim and research objectives

The overall aim of this research is to improve the risk assessment of human exposure to arsenic and its species through better defining the sources, pathways, and health impacts to support health risk management strategies. The data produced in this study on the contribution of dietary sources to species specific exposure, human biomarkers, health effects and potential health risks in six previously unstudied rural settings were used to address specific questions to provide a framework for improved risk assessment. The specific objectives and hypotheses addressed in this thesis are:

1.2.1 Objective-1: Characterize the potential sources of arsenic exposure

Characterisation of water and frequently consumed foods was performed, as these are the major arsenic exposure sources, to test the hypothesis that the study population was expected to consume more water per unit of body weight per day than international default or standard values and consequently be at higher health risk.

1.2.2 Objective-2: Identify the relative contribution of different arsenic species to arsenic exposure and human metabolism of these

Ascertaining the concentration of arsenic species in water, food and human biomarkers it was hypothesised that average daily intake of pentavalent iAs species will be higher than the trivalent iAs species and participants with arsenic induced health risks will show a higher capacity to methylate arsenic to MMA and a lower capacity to methylate MMA to DMA.

1.2.3 Objective-3: Health risk estimation through an integrated risk assessment approach

The ultimate objective is to integrate the improved knowledge of arsenic sources and human exposures to define the relative contribution of arsenic species to health risks. This will examine the hypothesis that trivalent iAs species will result in higher risk and unacceptable cancer risk will be found at drinking water iAs concentrations below 50 µg L^{-1}.

1.3 Thesis Outline

Chapter 1 provides a study rationale research gaps and outlines the aim and specific objectives to be addressed in this thesis. The rest of the thesis consists of nine chapters: two chapters based on a literature review and a summary of the methods used, five chapters of research manuscripts, one chapter synthesising the research and conclusions.

In **Chapter 2** a critical review of the arsenic risk assessment process is presented which includes an evaluation of the different exposure sources, exposure pathways and the relative hazards posed by different forms of arsenic. It also examines risk assessment models and techniques, associated variability and uncertainty and presents an update of the pertinent literature on the significance of integrated arsenic risk assessment. This highlighted the need for further research on the arsenic species in multimedia and how these relate to biomarker assessments of exposure.

Chapter 3 presents a generalized overview of the procedures for risk assessment and summarizes the overall methodological approach adopted in this thesis.

Chapter 4 addresses objective 1 by presenting the characterization of population specific water and food consumption patterns to prioritize the sources for exposure. Uniquely, the work evaluates the dietary differences across various regions and the validity of modelling As related health risks using population specific dietary data against generic values widely used in chemical risk assessments.

Chapter 5 is about arsenic speciation in the ground water sources of the study area to understand arsenic transport in the aquifers and available arsenic removal techniques along with their respective pros and cons. Addressing objectives 2 and 3, chronic exposure to arsenic species was assessed by comparing the individual exposure to reference values of daily dose.

Chapter 6 addresses arsenic speciation in staple foods (rice and wheat) and arsenic uptake by rice grains from cooking water. Addressing objectives 2 and 3, source and species specific exposure was assessed to estimate chronic health risk for every individual and to evaluate the possibility of achieving a preliminary advisory limit of iAs in rice.

Chapter 7 addresses objectives 2 and 3 by presenting the study outcomes based on the relative contribution of water and staple foods to arsenic intake and accumulation by multiple biological matrix measurements of inorganic and organic arsenic species in relation to potential modifier variables.

Chapter 8 addresses objective 2 by determining the potential relationships between arsenic exposure and skin disorders in the study area. It presents the dose-response association between arsenic exposure and the prevalence of skin lesions and the influence of confounding factors on this association.

Chapter 9 meets objective 3 by secondary analysis of the data presented in chapters 4 to 8 for an improved integrated risk assessment by determining population based risks of various types of health effects from intake of As and its species. The determined health risks were validated with bio-monitoring outcomes presented in chapters 8 and 9. Uniquely, the work evaluates the role of different arsenic species and not simply tAs on health risks.

Chapter 10 depicts the various conclusions drawn from this study and also defines the range of possible future work.

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Chapter 2: Human health risk assessment for arsenic: a critical review

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Abstract

Millions of people are exposed to arsenic resulting in a range of health implications. This paper provides an up-to-date review of the different sources of arsenic (water, soil and food), indicators of human exposure (biomarker assessment of hair, nail, urine and blood), epidemiological and toxicological studies on carcinogenic and non-carcinogenic health outcomes, and risk assessment approaches. The review demonstrates a need for more work evaluating the risks of different arsenic species such as; arsenate, arsenite monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid and dimethylarsinous acid as well as a need to better integrate the different exposure sources in risk assessments.

2.1 Introduction

Arsenic is a toxic and carcinogenic chemical (Pellizzari and Clayton, 2006, Hughes, 2006, International Agency for Research on Cancer, 2012b) that is a naturally occurring element and exists in the earth's crust at an average concentration of 5 mg kg^{-1} (Garelick et al., 2008). It is not, however, homogenously distributed in the crust and is more commonly associated with certain geological strata than others (National Academy of Sciences, 1977, Aronson, 1994). Whilst there are anthropogenic sources of arsenic, geological weathering is the primary cause of arsenic release into groundwater. This natural release of arsenic into ground or surface water poses a global public health risk for approximately 140 million people in at least 70 countries worldwide (Ravenscroft et al., 2009). Arsenic contaminated water also provides a pathway for arsenic to enter the food chain via irrigation as well as during food preparation and cooking (Bhattacharya et al., 2012, Fu et al., 2011, Mondal et al., 2010, Zavala and Duxbury, 2008, Zhao et al., 2010, Rahman et al., 2011a, Halder et al., 2014). Thus, ingestion of contaminated water

and food is a significant exposure pathway for arsenic. Long-term arsenic exposure has been associated with the development of skin lesions, various types of cancer, developmental effects, cardiovascular disease, neurotoxicity and diabetes (Steinmaus et al., 2013, Martinez et al., 2011).

Arsenic in water, food and soil exists in many different chemical forms and oxidation states (International Agency for Research on Cancer, 2012b) the most common inorganic and organic arsenic compounds found in water, food, soil and biomarkers referred to in this article are listed in Table 2.1.

Table 2.1: Inorganic and organic arsenic species

Arsenic type	Species	Abbreviation
Inorganic Arsenic (iAs)	Arsenate (arsenic acid)	AsV
	Arsenite (arsenous acid)	AsIII
Organic Arsenic	Monomethylarsonic acid or methylarsonic acid	MMAV
	Monomethylarsonous acid or methylarsonous acid	MMAIII
	Dimethylarsinic acid	DMAV
	Dimethylarsinous acid	DMAIII
	Arsenobetaine	AsB
	Arsenocholine	AsC
	Arsenosugars	-

Most of the trivalent and pentavalent arsenic species are absorbed in the body and transported via the blood stream to the body tissues (Capitani and Mello, 2011). Metabolism is mainly dependent on reduction-oxidation reactions causing inter-conversion of trivalent and pentavalent arsenic species and methylation of AsIII to yield methylated arsenic species. Generally, iAs forms are reported by Pal (2015) to be more toxic than organo-arsenicals. AsIII is considered comparatively more toxic than AsV, possibly due to interference of AsIII on enzymatic processes by bonding to sulfhydryl (–SH) or hydroxyl (–OH) functional groups (Kligerman et al., 2003, Mass et al., 2001, Hughes, 2002). Past studies have shown that trivalent methylated arsenicals are acutely more toxic and genotoxic than that of inorganic pentavalent arsenicals but the relative toxicity of individual arsenic species, such as MMAIII or DMAIII is still unknown (Tchounwou et al., 2003, Styblo et al., 2000, Viraraghavan et al., 1999). It has been suggested that the methylation of inorganic arsenic (iAs reduces) toxicity but data are conflicting (Petrick et al., 2000, Petrick et al., 2001). Therefore, there are still uncertainties regarding the potential risks and relative toxicity of individual arsenic species in the human body. This critical

review evaluates the current state of knowledge on the distribution and potential risks of different arsenic species from multiple exposure sources, through intake and uptake by the human body. It provides an overview of the associated health risks from environmental exposures, which can be used to eventually improve human health risk assessments.

2.2 Methodology: Literature search and selection strategy

A number of scientific publications databases: (Medline;PubMed), Environmental Sciences & Pollution Management *(*ESPM*),* the National Center for Biotechnology Information (NCBI) and University of Leeds Library Pro-quest were interrogated to identify peer-reviewed papers describing arsenic sources, exposure and risk, published between January 1961 and June 2015. An additional search was conducted on secondary literature such as books, reports and conference proceedings published around the world. Studies were selected based on the following selection criteria:

a. Concentrations reported for arsenic in surface and ground water, food items, soil, hair, nail, blood or urine.
b. Peer reviewed studies with methodological approach.
c. Potential health risks identified and associated to reported levels.
d. Risk estimates documented with variability and uncertainty.
e. Papers in English.

Of about 2000 items reviewed, 305 peer reviewed and published articles meeting the above criteria have been included in this review. In addition to the review, the relationships between tAs levels in water, soil, food and biomarkers identified in different studies reported across 22 countries (Tables 2.1-2.6) were evaluated using Pearson partial correlation analysis (SPSS 17.0, IBM, New York, NY, USA). Arsenic risk assessment techniques used for carcinogenic or non-carcinogenic risks estimates were also reviewed (Table 2.8) and critiqued to provide an overview of the current state of knowledge, knowledge gaps and further research needs.

2.3 Review Results

2.3.1 Arsenic origin and mobilization

Arsenic is categorized into three main exposure sources based on its origin and mobilization i.e. geological, anthropogenic and biological (Figure 2.1). Arsenic occurs in combination with arsenopyrite or sulphide in more than 150 minerals (Onishi and Sandell, 1955, Carapella, 1992, Budavari et al., 2013). In addition to naturally occurring arsenic deposits and sediments, other geological sources such as geothermal springs and volcanic ash are common (Bhattacharya et al., 2006a, Bundschuh et al., 2004, Nordstrom, 2002). Anthropogenic sources include metal mining and smelting which result in the release of arsenic sulphide (Straskraba and Moran, 1990). Other man made sources are the manufacture and use of pesticides (Tsuda et al., 1992, Mazumder et al., 1992, Matisoff et al., 1982, Tsuchiya, 1977), coal/wood burning, waste incineration, use in pharmaceutical and agricultural products/feeds, and electronics (US Environmental Protection Agency, 1998b, Sullivan, 1969). Many of these latter anthropogenic sources are now strictly controlled through regulation e.g. restrictions on use of copper chromated arsenate and other wood preservatives (Edelstein, 1985, European Economic Community, 2003).

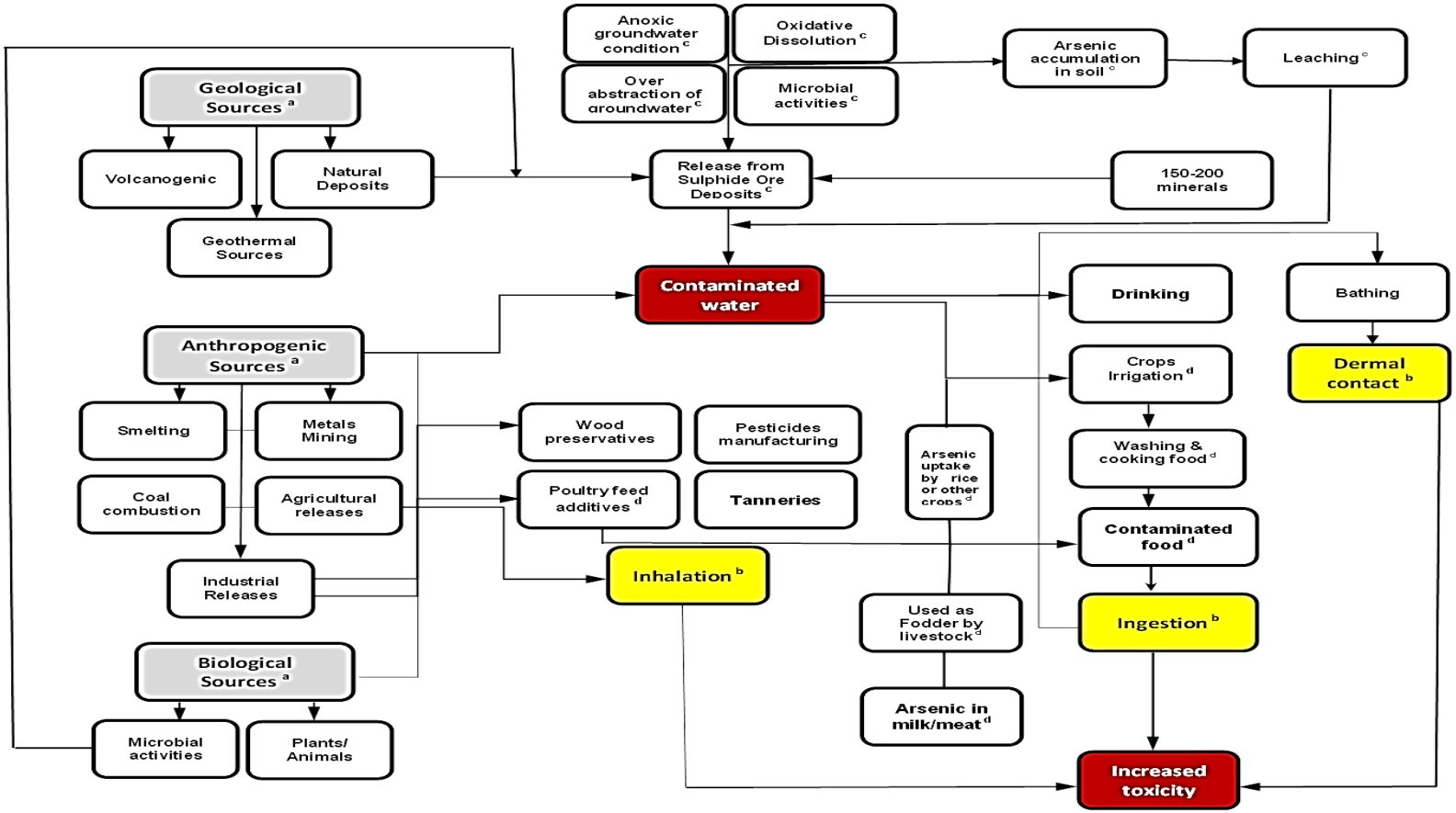

Figure 2.1: Arsenic sources, mobilization into water and food and exposure pathways:

a) Arsenic Sources-showing the release of arsenic from geological, anthropogenic and biological sources into ground water; b) Human exposure pathways through ingestion, inhalation and dermal contact; c) Mechanisms of arsenic mobilization into ground water hypothesized as arsenic adsorption by soil and its subsequent leaching into surface or ground water, arsenic release due to oxidation of pyrite or arsenopyrite, microbial and/or chemical reductive dissolution of iron oxyhydroxides, desorption and microbial mobilization, uncontrolled ground water abstraction and phosphate fertilizer; d) Arsenic enters the food chain from natural or anthropogenic sources and uptake by plants and crops from ground water used for irrigation.

Arsenic mobilization mechanisms from these different natural and anthropogenic sources include; arsenic adsorption by soil and its subsequent leaching into surface or ground water (US Environmental Protection Agency, 1998a, World Health Organization, 2001), oxidation of pyrite or arsenopyrite (Mallick and Rajagopal, 1996, Mandal et al., 1996), microbial and/or chemical reductive dissolution of arsenic-bearing iron oxyhydroxides in the aquifer sediments (Berg et al., 2008, Charlet and Polya, 2006, Zheng et al., 2004, Dowling et al., 2002), desorption and microbial mobilization (Garelick et al., 2008), uncontrolled ground water abstraction and application of phosphate fertilizer (Acharyya et al., 1999).

2.3.2 Arsenic in water

Arsenic mobilised from the aforementioned sources has been reported at concentrations up to 24000 µg L^{-1} in surface and groundwater sources (Table 2.2). The World Health Organization (1993) guidelines are 10 µg L^{-1} having been reduced from 50 µg L^{-1} in 1993, hence many regions around the world exceed the levels established for safe drinking water supplies.

Table 2.2: Arsenic levels reported in ground or surface water by mobilization source

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
Natural Geological	Loess deposits, thermal springs, holocene volcanic ash layer	Argentina	tAs: <1-14,969	tAs	2,750,000	Mukherjee et al. (2006a) Bundschuh et al. (2004) Claesson and Fagerberg (2003) Sifuentes and Nordberg (2003) Bates et al. (2004) Nordstrom (2002) Nicolli et al. (1989)
			tAs:7-14969 AsIII :1.2-1813 AsV :5.7-13156	Speciation based analysis		Bhattacharya et al. (2006b)
			AsIII : 1.2–8991	Speciation based analysis	9000	Smedley and Kinniburgh (2002)
	Pyritic sediments, increased groundwater abstraction	Australia	>10-7000	tAs		Appleyard et al. (2006)
	Alluvial sediments	Bolivia	>10-964	tAs		Johnsson and Wern (2010) Van den Bergh et al. (2010)
	Older alluvial, Holocene, Pleistocene and Fluvio sediments, Microbial mediated degradation of organic matter and reductive dissolution of Fe-oxyhydroxide	Bangladesh	>50-4700	tAs	35-79 million	van Geen et al. (2014) Halim et al. (2009) Tareq et al. (2003) Chowdhury et al. (2000) Nickson et al. (2000) Smith et al. (2000) Chowdhury et al. (1999) Dhar et al. (1997) Khan and Ahmad (1997)

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
						Bristish Geological Survey and the Department of Public Health Engineering (2001)
	inter-dune lake sediments	Brazil	>50	tAs		Mirlean et al. (2014)
	Volcanic rocks Sulfide ore deposits Weathering products at the Andean volcanic chain Geothermal manifestations	Chile	750-800	tAs	130,000-400,000	Dougnac (1999) Bundschuh et al. (2009) Landrum et al. (2009) Romero et al. (2003) Smith et al. (1998)
	Geological Arsenic ore reserves Spatial distribution of Fe oxides Natural; alluvial and lake sediments; high alkalinity	China	>50-2400	tAs	3.0 million	He and Charlet (2013) Yu et al. (2007) Sun G-F (2004) Jin et al. (2003) Smedley et al. (2003) Nordstrom (2002) juan Guo et al. (2001)
	Holocene sediments at depths >16 m Mekong and Bassac river channels.	Cambodia	0.21–1700	tAs	0.5–1 million	Gault et al. (2008) Berg et al. (2007) Polya et al. (2005)
	Proterozoic volcanic sedimentary rocks	Finland	17-980	tAs	9000	Kurttio et al. (1999)
	Numerous volcanoes, hot springs, fumaroles, and geothermal wells	El Salvador	10-770	tAs		López et al. (2008) López et al. (2012)
	geological	Ethiopia	<1-70	tAs		Merola et al. (2014a)
	Geothermal deltaic sediments hydrothermal activities	Greece	1- 3760	tAs		Casentini et al. (2011) Kouras et al. (2007)

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
	Deeper anoxic waters					Katsoyiannis and Katsoyiannis (2006)
	specific lithofacies sediments	Germany	<10-150	tAs		Heinrichs and Udluft (1999)
	volcanic rocks	Guatemala	1-15	tAs		Dougnac (1999)
	Geothermal springs	Honduras	70-1260	tAs		Fraser et al. (1986)
	alluvial sediments and arsenic rich organic material	Hungary	4-310	tAs	33,006	Lindberg et al. (2006) Varsanyi et al. (1991) Varsányi (1989) Varsanyi et al. (1991)
	Geological	Nepal	>10-50	tAs	0.5 million	Yadav et al. (2012) Gurung et al. (2007) Shrestha et al. (2003) Tandukar (2001)
	Geothermal outflow from Volcán Telica volcanic rocks	Nicaragua	>10	tAs	1000	Longley (2010) McClintock et al. (2012) Jorge Mendoza Aldana (2010)
	Geological	Mayanmar	>10	tAs	03 (cases of Arsenicosis)	Tun (2003)
	Geological and Quaternary volcanic Activity	Iran	11-1480	tAs		Keshavarzi et al. (2011) Mosaferi et al. (2003)
	Geothermal sources	New Zealand	9.8-8500	tAs		Wilson and Webster-Brown (2009) Robinson et al. (1995) Ritchie (1961)
	Geological	Pakistan	>10-2400	tAs	2.0 millions	Tahir and Rasheed (2014) Ahmed et al. (2014) Malana and Khosa (2011) Toor and Tahir (2009) Farooqi et al. (2007)

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
						Nickson et al. (2005) Kahlown et al. (2005) Ahmad (2004)
	Geological	Romania	46.36 -179.98	tAs	41,000	Gurzau and Pop (2012) Mukherjee et al. (2006b)
	Geological	Serbia	5-420	tAs		Stanisavljev et al. (2013) Jovanovic et al. (2011)
	Arsenic containing ore and sediments	Switzerland	>10-170	tAs		Pfeifer and Zobrist (2002)
	Arsenopyrite waste piles alluvial deposits	Thailand	1.25- 9000	tAs	15000	Kohnhorst et al. (2002) Williams et al. (1996) Fordyce et al. (1995)
	Geological	Taiwan	tAs: <0.15-3,000	tAs	40,421 in 37 villages	Chen et al. (2010a) Mukherjee et al. (2006b)
	Geological	Taiwan	AsIII : 318-683 AsV : 33-420 MMA: <1 DMA: <1	Speciation based analysis	1141 patients	Chen et al. (1994)
	Anoxic groundwater iron oxy-hydroxides sediments	Vietnam	>10-3050	tAs	1 million	Merola et al. (2014a) Nhan et al. (2013) Winkel et al. (2011) Berg et al. (2008) Berg et al. (2001)
	sediments containing volcanic ash	Uruguay	18-30	tAs		Dougnac (1999)
Anthropogenic sources	Smelter unit processing sulphide ores	Bulgaria	750-1500	tAs		Nilsson et al. (1993)
	Gold mines	Cuba	25-250	tAs		Toujaguez et al. (2013)

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
	Contaminated ballast water from old oil terminal, mine waters from the Cerramotoso nickel mine	Colombia	60-690	tAs		Mazo-Gray et al. (1997)
	Gold mining	Ecuador	390-670	tAs		Cumbal et al. (2010)
	Gold mining	Ghana	tAs: <1-175 AsIII : <3	Speciation based analysis	100,000	Smedley (1996)
Combination of geological and anthropogenic sources	Fluvial inputs originating from the Deloro mining site Organic, marine and glaciomarine sediments	Canada	22-75	tAs	27	Meranger et al. (1984) Azcue and Nriagu (1995) Zheng et al. (2003) Wilson et al. (2008)
	Geological as arsenic rich sediment i.e Holocene, alluvia/delltaic sediments with high phosphate or organic matter deposits arsenical pesticides	India	10-5800	tAs	100 million	Chakraborti et al. (2003) Srivastava and Sharma (2013) Yano et al. (2012) Chakraborti et al. (2003) Chakraborti et al. (2009) Mukherjee et al. (2006b) Rahman (2005) McArthur et al. (2004) Nordstrom (2002) Smedley and Kinniburgh (2002) Mandal et al. (2003) Chowdhury et al. (2000) Pandey et al. (1999) Das et al. (1996) Das et al. (1995) Mazumder et al. (1992) Hoque et al. (2012)

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
						Acharyya (2002)
	Geological, mining Industrial waste containing arsenic sulphide, arsenical containing insecticides	Japan	1-293	tAs	18 (deaths from cancer)	Mandal and Suzuki (2002) Tsuda et al. (1992) Tsuchiya (1977) Mukherjee et al. (2006a)
	Alluvial sediments Mining activities Over abstraction of ground water	Mexico	tAs: 14-24000	tAs	450,000	Dougnac (1999) Aldo Uriel et al. (2013) Armienta et al. (2001) Rosas et al. (1999) Gómez-Arroyo et al. (1997) Del Razo et al. (1990) Armienta et al. (1997)
			iAs: 3.12-319 AsIII : 0.25-5.12 AsV : 3.12-315	Speciation based analysis		Rosas et al. (1999)
	Mining and volcanic rock formations	Peru	>10-400	tAs	250,000	George et al. (2014) Dougnac (1999) de Esparza (2008)
	Geological, mining and smelting	United Kingdom	11-5000	tAs		Middleton et al. (2016) Aston et al. (1975)
	Geologic land use practices, volcanic rocks, bedrock wells gold and coal mining arsenical pesticides	USA	<1-1300	tAs	35000-285,000	James et al. (2014) Peters et al. (2006), US Geological Survey (2003) US Geological Survey (2003) Welch et al. (2000) Lewis et al. (1999) Brown and Fan (1994) Matisoff et al. (1982) Wilson and Hawkins (1978) Robertson (1989)

Source	Type	Country	Average As concentration (µg L^{-1})	Arsenic testing as	Population at risk or affected (persons)	Reference
	arsenic rich abandoned mine dumps	Zimbabwe	13-96	tAs		Jonnalagadda and Nenzou (1996)
Not Known		Afghanistan	>10-500	tAs	500,000	Mukherjee et al. (2006a)

High arsenic levels have been reported in Argentina, Australia, New Zealand, Mexico, India and Thailand (Figure 2.2). However, the highest levels of arsenic in water resources reported were for Bangladesh and India, where nine districts in West Bengal, India, (Chowdhury et al., 2000) and 59 districts in Bangladesh had arsenic levels in excess of the WHO guideline value (10 µg/l) (Chakraborti et al., 2010). About 20,000 deaths per year in Bangladesh have been attributed to exposure to arsenic, whereas an estimated 50 million people are considered at risk of health consequences (Pearce, 2001, Chaudhuri, 2004).

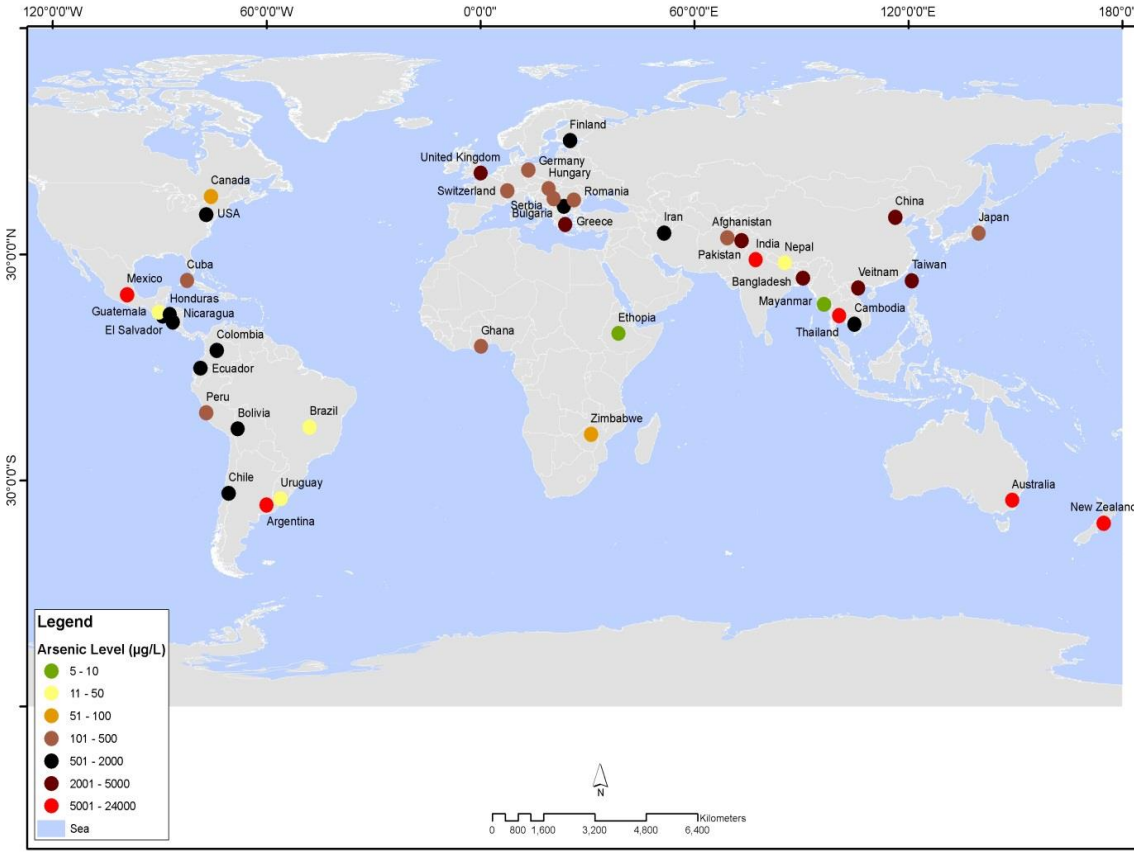

Figure 2.2: Global distribution of arsenic in water indicated by GIS (Geographical Information System) characterisation of levels of arsenic in water sources of 43 countries. Lowest range up to WHO guideline of drinking water ≥10 µg L-1 indicated by green circle and highest level by red circle. See Table 2.2 for all references.

Most studies assessing arsenic concentrations in water (Table 2.2) have evaluated tAs levels with relatively few considering the different arsenic species. It is assumed that methylated-arsenic compounds are low in ground water unless special circumstances, such as pollution by arsenical herbicides or high biological activity, exist (Welch et al., 2000). Irgolic (1994) concluded that methylated species

(MMA and DMA) would rarely be present in water supplies and thus their determination in water is unnecessary for regulatory purposes. There are a small number of studies that have evaluated arsenic species in water, particularly regarding the mobilisation from underlying geology to groundwater. For instance, Bhattacharya et al. (2006b) reported concentrations of AsIII and AsV in groundwater from geological sources, whilst Smedley and Kinniburgh (2002) analysed aquifer pore waters for AsIII and AsV. Earlier work by Smedley (1996) looked at AsIII and AsV in groundwater in aquifers in Ghana, whereas, Rosas et al. (1999) examined the relationship between arsenic species (tAs, AsIII, AsV) in soil and water. Chen et al. (1994) attempted to go one stage further by measuring AsIII, AsV, MMA and DMA in water and linking it to human health outcomes with limited success. Understanding the metabolic fate and relative toxic effects of various chemical forms of arsenic may remain incomplete without drinking water source characterisation and exposure assessment of arsenic species.

2.3.3 Arsenic uptake by plants from soil and irrigation practices

Arsenic distribution in soils is reported within a widely variable range up to 43,500 mg kg^{-1} (Table 2.3). Arsenic above the European Union (EU) recommended maximum acceptable limit for agricultural soil such as 20 mg kg^{-1} (Rahman et al., 2007) has been associated with mining activities (Zhu et al., 2008), contaminated groundwater used for irrigation (Meharg and Rahman, 2003) and use of arsenical pesticides (Williams et al., 2007) as summarised in Figure 2.1.

Table 2.3: Summary of arsenic distribution in soil

Possible source	Reported arsenic levels (mg kg^{-1})	Arsenic testing as	Reference
Geological	5.0	tAs	Reichert et al. (1921)
	0.32-18	tAs	Mäntylahti and Laakso (2002) RAKAS Project (2007)
	0.50-22.9	tAs	Wei et al. (1991)
	2.9-41.7	tAs	Phuong et al. (2008)
	10-46	tAs	Meharg and Rahman (2003) Rahman et al. (2011b)
	9.38-57.1	tAs	Ong et al. (2013)
	6.5-65	tAs	Slekovec and Irgolic (1996)
	11-30	tAs	Rosas et al. (1999)
	10-196	tAs	Roychowdhury et al. (2002)

Possible source	Reported arsenic levels (mg kg^{-1})	Arsenic testing as	Reference
			Chakraborti et al. (2002)
	0.8-500	tAs	Seyfferth et al. (2014) Kocar and Fendorf (2012)
Geothermal sources	40–116	tAs	Flores-Tavizón et al. (2003)
Mining and tailing	2.1-183	tAs	Skála et al. (2011)
	4 to 14,700	tAs	Ongley et al. (2007)
	5.3-2035	tAs	Baroni et al. (2004)
	11.4-439	tAs	Norton et al. (2013)
	13-64 (as sum of tAs, AsIII and AsV)	Speciation based analysis	Acosta et al. (2015)
	34-1198	tAs	Pfeifer and Zobrist (2002)
Multiple sources: (geological, gold and copper mining, sulphide mineralization, pesticides application, industrial disposal of arsenopyrite (FeAsS), offshore oil fields and industrial waste)	0.72-38.2	tAs	Iimura (1978) Arao et al. (2010)
	0.8-99.5	tAs	Overesch et al. (2007)
	1-3000	tAs	Wenzel et al. (2002)
	1.21-56.17	tAs	Weerasiri et al. (2014) Weerasiri et al. (2013) Srinuttrakul and Yoshida (2013)
	1.8-830	tAs	Pettry and Switzer (2001) Smith et al. (1998)
	1.8-60	tAs	Ungaro et al. (2008)
	6.13-89.2	tAs	Ghani et al. (2013)
	22-157	tAs	Amonoo-Niezer and Busari (1979)
	100-43,500	tAs	Krysiak and Karczewska (2007)
	280.3-1207.4	tAs	Bidone et al. (2014)
	tAs: 9400-13500 AsIII :<2-504 AsV :4921-10504 MMA: <2 DMA:<2	Speciation based analysis	Matera et al. (2003)

Arsenic contamination of soil by irrigation water and subsequent uptake by crops poses a potentially significant public health risk. There are relatively few studies that have identified a positive correlation between arsenic concentrations in soil and irrigation water (Meharg and Rahman, 2003, Duxbury and Zavala, 2005, Das et al., 2004), and between arsenic uptake by rice and arsenic in soil water (Loeppert et al., 2005, Meharg and Rahman, 2003). Moyano et al. (2009) have shown that potatoes irrigated with arsenic-rich water have 35 times more arsenic compared with other crops. They have also confirmed the uptake of arsenic from contaminated irrigation water by beet, carrot and wheat crops. As for water, most

monitoring studies have focused on total arsenic (tAs) with few looking at the individual arsenic species present. Studies that have measured arsenic species in soils have reported higher levels of the less toxic AsV compared to AsIII (Acosta et al., 2015, Matera et al., 2003). Similarly, Smith et al. (2008) have demonstrated that root, shoot and leaf tissues contained mainly inorganic AsIII and AsV species, while rice grains contained predominantly DMA (85 to 94%) and AsIII. Generally, there are few studies that evaluate the quantification of the influence of arsenic contaminated irrigation water, accumulation of arsenic in top soils, land degradation pattern, relationship between water-soil-plant system and risks of arsenic contaminated irrigation water to crop production, specifically from the perspective of arsenic species.

2.3.4 Arsenic in the food chain

Evidence suggests that arsenic uptake by plants varies (Sharma et al., 2014), influenced by the water requirements of different crop types, levels of soluble arsenic species in soil, soil properties, redox and pH conditions, microbial activity, and plant species (Norra et al., 2005, Lehoczky, 2002). Arsenic can accumulate in the food chain if herbivorous animals are fed diets rich in arsenic-contaminated feedstock or drink from arsenic-contaminated water supplies. For humans, the main food sources have been suggested to be fish, crops (rice, cereals), vegetables, fruit, poultry and animal products (meat and milk) (Table 2.4).

The WHO has established a guideline permissible limit value of 0.1 mg kg^{-1} tAs in food which is frequently exceeded by many of the food groups that have been analysed (Table 2.4). Total arsenic detected in various food categories fall in the range of not detected to 1.9 mg kg^{-1} for cereals, 13 mg kg^{-1} for vegetables, 22.4 mg kg^{-1} for fruits and fruit juices, 42.6 mg kg^{-1} for animal products and 98 mg kg^{-1} for fish and sea food. Rice, however, demonstrates the highest levels of arsenic in food with the maximum level reported at 267.7 mg kg^{-1} (Nookabkaew et al., 2013). Rice is an efficient scavenger of arsenic and takes up ten times as much as other cereal crops probably due to growth in flooded fields (Sohn, 2014, Wang et al., 2013, Khan et al., 2010, Meharg et al., 2009, Zavala and Duxbury, 2008). As such, arsenic exposure is likely to be greater for people who eat large amounts of rice every day and for infants, whose first solid meals are mainly rice-based baby food.

The relative toxicity of arsenic in foods depends on its chemical form and bioaccessibility (Juskelis et al., 2013). In contrast to water, arsenic species have been well studied in food items with both organic and inorganic species identified in a range of food items, from milk to fish and rice (Carey et al., 2010b, Meharg et al., 2009, Zavala and Duxbury, 2008, Norton et al., 2013, Schoof et al., 1999a, Jackson et al., 2012, Meharg et al., 2008, Li et al., 2003) (Table 2.4). Studies have generally reported higher levels of toxic inorganic forms such as arsenite (AsIII) rather than the more mobile inorganic arsenate (AsV) and organic species.

Table 2.4: Summary of arsenic distribution in food items

Food item	**Type**	**Reported levels (mg kg^{-1})***	**Arsenic testing as**	**Reference**
Rice	White rice (small-long grains)	0.01	tAs	US Food Drug Administration (2013)
	Polished (white) grain rice	tAs: 0.5-85.2	tAs	Wang et al. (2013) Khan et al. (2010)
		tAs:0.05-0.28 AsIII : 0.049-0.572 AsV : <0.005-0.095 DMA: 0.04-0.572	Speciation based analysis	Carey et al. (2010a) Meharg et al. (2009) Zavala et al. (2008)
	Cooked rice	0.057	tAs	Khan et al. (2010)
	Boro rice grain	0.45	tAs	Bhattacharya et al. (2010)
	White rice	86.5–115.9	tAs	Nookabkaew et al. (2013)
	Brown rice	203.7–267.7	tAs	Nookabkaew et al. (2013)
Cereals	Corn (*Zea mais*)	0.004-1.9	tAs	Muñoz et al. (2002) Queirolo et al. (2000) Schoof et al. (1999b)
	Wheat flour	<0.05-0.01	tAs	Schoof et al. (1999b) Liukkonen-Lilja (1993)
	Grains and pulses	0.016	tAs	Sancha and Marchetti (2008)
	Rye flour	<0.02	tAs	Liukkonen-Lilja (1993)
Vegetables	Peas	0.005	tAs	Schoof et al. (1999b)
	Cucumber	0.004	tAs	Schoof et al. (1999b)
	Beet sugar	0.004	tAs	Schoof et al. (1999b)
	Spinach	0.02	tAs	Schoof et al. (1999b)
				Khan et al. (2010)
	Potato	0.01-0.86	tAs	Norton et al. (2013) Bhattacharya et al. (2010) Queirolo et al. (2000)
	Turmeric	0.003	tAs	Bhattacharya et al. (2010)
	Chili (*Capsicum*)	8.0	tAs	Prieto-García et al. (2005)
	Chayote squash (*Sechium edule*)	5.1	tAs	Prieto-García et al. (2005)
	Amaranth	0.023	tAs	Khan et al. (2010)
	Cabbage	0.02	tAs	Wang et al. (2013)
	Cauliflower	0.01-0.06	tAs	Muñoz et al. (2002)

Food item	Type	Reported levels (mg kg^{-1})*	Arsenic testing as	Reference
	Onion	0.35–5.4	tAs	Institute of Food Technology (2006)
	Carrots	3.8	tAs	Institute of Food Technology (2006)
	Yam roots	4.8	tAs	Palmieri et al. (2009)
	Bean grains	8.3	tAs	Palmieri et al. (2009)
	Broad beans	2.3- 2.9	tAs	Institute of Food Technology (2006)
	Salad, mix	0.06	tAs	Norton et al. (2013)
	Lettuce leafs	tAs: 13 AsIII : 0-30.6 AsV : 39.6-1913.9 MMA: 0-5.5 DMA: 0-24.3	tAs & speciation based analysis	Norton et al. (2013)
Fruits and Fruit juices	Currants	0.012	tAs	Norton et al. (2013)
	Grape juice	tAs: 0.009 µg L^{-1} AsIII : 2.60-35.65 AsV : 2.06-15.30 MMA: <0.04-0.25 DMA: 0.27-2.07	Speciation based analysis	Schoof et al. (1999b)
	Apple cider	tAs: 5.41-15.27 µg L^{-1} AsIII : 0.98-4.29 AsV : 2.90-11.20 MMA: 0.80-0.81 DMA: 0.30-0.92	Speciation based analysis	Roberge et al. (2009)
	Apple juice	10.8-22.4 µg L^{-1}	tAs	Jackson et al. (2012)
	Pear containing products	0.017	tAs	Jackson et al. (2012)
	Oil palm fruit	4.53	tAs	Amonoo-Neizer and Amekor (1993)
	Cane sugar	0.004	tAs	Schoof et al. (1999b)
Animal products	Raw milk	0.42-9.13 µg L^{-1}	tAs	Pérez-Carrera and Fernández-Cirelli (2005)
	Whole milk	tAs: 2.78-7.92 µg L^{-1}* AsIII : <0.05-0.94 AsV : 0.28-1.05 MMA: <0.04 DMA: <0.04	Speciation based analysis	Roberge et al. (2009)
	Chicken broth	tAs: 11.1-22.8 µg L^{-1}* AsIII : 0.17-1.38 AsV : <0.06-0.78 MMA: <0.04 DMA: <0.04	Speciation based analysis	Roberge et al. (2009)
	Beef broth	tAs: 19.1- 42.6 µg L^{-1} AsIII : 1.14-5.94 AsV : 0.37-6.56 MMA: <0.04 DMA: <0.04-0.17	Speciation based analysis	Roberge et al. (2009)
	Peanut butter	0.005	tAs	Schoof et al. (1999b)
	Eggs	0.0642	tAs	Schoof et al. (1999b)
Baby foods	Infant formulas and first foods	tAs: 0.02–0.013 µg L^{-1} DMA: 19-40 µg L^{-1}	Speciation based analysis	Jackson et al. (2012)
	Baby rice	tAs:0.15-0.47 DMA: 0.03-0.23	Speciation based analysis	Meharg et al. (2008)

Food item	Type	Reported levels (mg kg^{-1})*	Arsenic testing as	Reference
Fish and Sea food	Fresh water fish	tAs :0.02-15.8	tAs	Wang et al. (2013) Liang et al. (2013) Liang et al. (2013) New South Wales Food Authority (2010) Moreno Lopez (2008) Stassen and van de Ven (2007) Mora et al. (2001) Quevillon et al. (1996) Amonoo-Neizer and Amekor (1993)
	Fresh water fish	TAs :0.26-2.38 DMA: 0.045 AsB:0.13-1.73	Speciation based analysis	Li et al. (2003)
	Blue Shark	8.0	tAs	Macedo (2010)
	Atlantic Cod Fish (Haddock)	11.4	tAs	Julshamn et al. (2004)
	Prawns	62	tAs	Julshamn et al. (2004)
	Shell Fish	tAs: 0.24-0.37 DMA: LOD AsB: 0.15-0.24	Speciation based analysis	Li et al. (2003)
	Crustaceans	tAs: 0.45-7.54 DMA: LOD-0.029 AsB: 0.34-6.60	Speciation based analysis	Li et al. (2003)
	Hijiki Seaweed	77	tAs	Food Standards Agency (2004)
	Sea Weeds	39.0	tAs	New South Wales Food Authority (2010)
	Mollusc Specie (*Lapa Negra*)	1.17-6.07	tAs	Dougnac (1999)
	Fresh Water Algae	98	tAs	Díaz et al. (2008)
	Blue Mussels	3-15.8	tAs	Sloth and Julshamn (2008)

*for beverages/liquid foods, the concentration unit is µg L^{-1}

2.3.5 Human exposure pathways and bioavailability

Humans can be exposed to arsenic through a variety of exposure routes. Airborne arsenic released from industrial emissions result in occupational exposure through inhalation (US Public Health Service, 1989). For instance, peripheral neuropathy among smelter workers has been linked to exposures above the WHO air quality limit of 1 µg m^{-3} arsenic (Lagerkvist and Zetterlund, 1994). Releases of 20 to 760 µg m^{-3} airborne arsenic associated with the burning of arsenic-rich coal in China have resulted in 3,000 patients with skin lesions on the hands and feet, pigmentation on the trunk, skin ulceration, and skin cancers (Liu et al., 2002).

Dermal contact, which might result from washing in contaminated water and/or handling products containing arsenic (e.g. wood preservatives), has also been suggested as a pathway of exposure but few studies have evaluated this in detail (Roels et al., 1980, Pirnie Malcom Inc, 2001, Galarneau et al., 1990). The ingestion of arsenic through drinking water, using contaminated water in food preparation, irrigation of food crops, food or beverage industrial processes and eating contaminated food are considered to be the primary exposure pathways (Tsuda et al., 1992). Water has long been considered the main exposure route for arsenic, with levels of AsIII or AsV influenced by pH, redox potential or salinity of the water body (Smedley and Kinniburgh, 2002). Different opinions on the overall exposure contribution of arsenic in food exist. For example, a US study on arsenic toxicity concluded that iAs exposure through food does not pose higher risks of carcinogenicity (Boyce et al., 2008). Meharg et al. (2009), however, assessed the health risks arising from consumption of arsenic-contaminated white rice; using country-specific rice consumption data for five countries, they reported an excess of cancer linked to inorganic arsenic (iAs) from 0.7 per 10,000 population in Italy to 22 per 10,000 in Bangladesh – almost a 30-fold increase in cancer risk. This is further supported by other studies, which suggest an association between arsenic in food and increased cancer risk (Meacher et al., 2002, Schoof et al., 1999b).

Linking exposure with potential health impacts depends on arsenic intake and uptake, which may be affected by type (inorganic or organic) and concentration of trivalent (AsIII, MMAIII and DMAIII) or pentavalent arsenic forms (AsV, MMAV and DMAV) found in water or food, and how these different arsenic species are processed by the human body. In the human biological environment, AsIII and AsV are considered comparatively more toxic than methylated organic (MMAV and DMAV) forms (Abedin et al., 2002, Meharg and Hartley-Whitaker, 2002). Quantification and risk assessment approaches may prove useful to understand the differences between individual arsenic species and person-to-person variation. People within a community or household sharing the same drinking water source may not be equally affected and show variable clinical manifestations (Huq and Naidu, 2004). This might be due to confounding factors such as nutritional deficiencies, low selenium intake, smoking and genetic factors, all of which have been observed to enhance the development of arsenicosis (Deb et al., 2013, Chen

et al., 2001, Gamble et al., 2007, Spallholz et al., 2004, Miyazaki et al., 2005, Lamm et al., 2006, Lamm and Kruse, 2005). The influence of these variables on the toxicity levels of various chemical forms of arsenic is yet to be explored in any detail.

2.3.6 Metabolic pathways and biomarkers of exposure

Arsenic metabolism within the human body is dependent on the inter-conversion of AsIII and AsV. About 40-100% of tAs is absorbed as AsV from the human gastrointestinal tract (Saha et al., 1999). AsIII can bind to bioactive protein molecules (National Research Council, 1999) but is less likely to be absorbed than soluble inorganic forms in water (European Food Safety Agency, 2009). Whilst all the processes involved in the metabolism of iAs have not been fully elucidated, an overall metabolic pathway for arsenic (Equation 2.1) has been proposed (Thomas et al., 2001, McKinney, 1992, Thompson, 1993).

$$As^{III} \xleftrightarrow{Oxidation/reduction} As^{V} \xrightarrow{methylation} MMA^{V} \xrightarrow{reduction} MMA^{III} \xrightarrow{methylation} DMA^{V} \xrightarrow{possible\ reduction} DMA^{III} \quad (Eq.\ 2.1)$$

(Simplified model of arsenic metabolism)

Certainly, metabolism of arsenic has a role in this effect. As a proxy to understanding this role, human biomarkers have been used as indicators. Biomarkers are quantifiable changes in biochemical, physiological or behavioural states within cells, tissues or whole individuals because of external stressors (Timbrell, 2002). Biomarkers are classified as markers of exposure, effect, or susceptibility (National Research Council, 1989) and provide useful information on fate and metabolism of arsenic within human body. To evaluate the metabolic process and fate of arsenic within human body, samples of hair, nail, blood and urine have been examined for traces of arsenic (Tables 2.5-2.6). It has been suggested that arsenic accumulates in hair and fingernails due to preferential binding to proteins such as keratin (National Research Council, 1999). Biomarker analysis of hair and nails can therefore be used to confirm arsenic intake and associated accumulation of arsenic in the human body (Table 2.5). The highest level reported in hair is 1,500 mg kg^{-1} (Concha et al., 2010) whilst for nails it is 5406

µg kg^{-1} (Button et al., 2009) and urine 1000-6200 µg L^{-1} (Lindberg et al., 2006): blood reveals the lowest levels of 1-14.3 µg L^{-1}.

Table 2.5: Summary of human studies measuring biological arsenic in hair, nail and blood

Biomarker type	Reported level	Unit*	Arsenic testing as	References
Hair	1.6-4.64	mg kg^{-1}	tAs	Rahman et al. (2006)
	2-5 (exposed cancer patient)	mg kg^{-1}	tAs	Wadhwa et al. (2011)
	0.10–4.57	mg kg^{-1}	tAs	Aldroobi et al. (2013)
	0.018–1.0	mg kg^{-1}	tAs	Normandin et al. (2014)
	4.2	mg kg^{-1}	tAs	Cui et al. (2013)
	nd-0.38	mg kg^{-1}	tAs	Intarasunanont et al. (2012)
	0.01-57.21	mg kg^{-1}	tAs	Phan et al. (2011)
	2002: 0.48-10.83 2006: 0.27-8.25	mg kg^{-1}	tAs	Wu and Chen (2010)
	0.27-23.85	mg kg^{-1}	tAs	Pereira et al. (2010)
	0.0059-0.0644	mg kg^{-1}	tAs	*Essumang (2009)*
	0.20 to 6.50	mg kg^{-1}	tAs	Gault et al. (2008)
	0.088-2.77	mg kg^{-1}	tAs	Agusa et al. (2006)
	20–1,500	mg kg^{-1}	tAs	Concha et al. (2010)
	4.20	mg kg^{-1}	tAs	Yanez et al. (2005)
	tAs: 0.07-4.61 AsIII 0.21-2.64 DMAV: :0.02-0.13 MMAV: : 0.02-0.2 AsV :0.08-1.54	mg kg^{-1}	Speciation based analysis	Mandal et al. (2003)
	5.52	mg kg^{-1}	tAs	Hinwood et al. (2003)
	0.2-5.60	mg kg^{-1}	tAs	Pazirandeh et al. (1998)
	<0.006-0.582	mg kg^{-1}	tAs	Gebel et al. (1998)
	1.18-31.05	mg kg^{-1}	tAs	
	0.43-5.74	mg kg^{-1}	tAs	Harrington et al. (1978)
Nails	Significant correlation between Arsenic in drinking water and nails (r = 0.49, *P*<0.001)	mg kg^{-1}	tAs	Merola et al. (2014a)
	0.61-27.89	mg kg^{-1}	tAs	Rahman (2005)
	Significant correlation between arsenic in toenails and drinking water	mg kg^{-1}	tAs	Merola et al. (2014b)
	0.19	mg kg^{-1}	tAs	Cottingham et al. (2013)
	0.008–1.4	mg kg^{-1}	tAs	Normandin et al. (2014)
	7.8	mg kg^{-1}	tAs	Cui et al. (2013)
	0-8.23	mg kg^{-1}	tAs	Intarasunanont et al. (2012)
	Finger nail: 0.03-28.47 Toenail: 0.10- 21.89	mg kg^{-1}	tAs	Phan et al. (2011)
	0.10 to 7.95	mg kg^{-1}	tAs	Gault et al. (2008)
	tAs: 5406 AsIII 11477 DMAV: 84 MMAV: 73 AsV 2899	µg kg^{-1}	Speciation based analysis	Button et al. (2009)
	0.02 to 2.11	mg kg^{-1}	tAs	Michaud et al. (2004)

Biomarker type	Reported level	Unit*	Arsenic testing as	References
	2.94	mg kg^{-1}	tAs	Wilhelm et al. (2004) Wilhelm et al. (2005)
	tAs: 1.47-7.39 AsIII 0.95-2.76 MMAIII : 0.09-0.21 DMAIII 0.11-0.38 DMAV: 0.04-0.09 AsV 0.27-1.31	mg kg^{-1}	Speciation based analysis	Mandal et al. (2003)
	21.7	mg kg^{-1}	tAs	Hinwood et al. (2003)
	<0.01 to 0.81	mg kg^{-1}	tAs	Karagas et al. (2000)
	1.47-52.03	mg kg^{-1}	tAs	Das et al. (1995)
	4 (in 37% of persons)	mg kg^{-1}	tAs	Harrington et al. (1978)
Blood	3.29-8.82 (exposed cancer patients)	µg L^{-1}	tAs	Wadhwa et al. (2011)
	1.31-10.37 (new borne blood)	µg L^{-1}	tAs	Intarasunanont et al. (2012)
	14.3	µg L^{-1}	tAs	Hall et al. (2006)
	1.0-18.3	µg L^{-1}	tAs	Vahter et al. (1995)

There have been fewer arsenic speciation analyses carried out for hair and nails compared to urine possibly due to the more complex sample preparation required to remove contaminants adsorbed to the surface of the collected materials (Hindmarsh et al., 1999, Mandal et al., 2003, Button et al., 2009). Urinary arsenic metabolites have been used to correlate arsenic exposure with arsenic intake rates, arsenic methylation mechanism, human bioaccumulation and excretion capacity and to determine carcinogenic or non-carcinogenic health impacts. Urinary metabolites studies (listed in Table 2.6) have indicated that most of the ingested arsenic is methylated and excreted as DMA (79–85%), with smaller amounts excreted as iAs (8–16%) or MMA (5–6%) (Christian et al., 2006).

Table 2.6: Summary of human studies measuring biological arsenic in urine

Reported levels	Unit	Arsenic testing as	References
Exposed: 6.6 Unexposed: 5.0	µg L^{-1}	tAs	Neamtiu et al. (2015)
Males: 124 Females: 130	µg L^{-1}	tAs	Mazumder et al. (2013)
AsIII: 0.03-7.38 DMAV : 0.32-7.38 MMAV : 0.03-31.5 AsV : 0.03-13.3	µg L^{1}	Speciation based analysis	Normandin et al. (2014)
56.0 (sum of arsenic species)	µg L^{-1}	speciation based analysis	Cui et al. (2013)
117 ± 8.3	µg g^{-1} of creatinine**	tAs	Liu et al. (2013)

Reported levels	Unit	Arsenic testing as	References
AsIII: 16.8 AsV: 1.8 MMA: 1.8 DMA: 88.6	µg L^{-1}	Speciation based analysis	Hata et al. (2012)
15	µg g^{-1}	tAs	Robles-Osorio et al. (2012)
Maternal urinary creatinine: 0-0.43	µg $mmol^{-1}$ (creatinine, lower than reference background level of 28 µg $mmol^{-1}$ creatinine)	tAs	Intarasunanont et al. (2012)
tAs: 19.1 (AsIII+AsV+MMA+ DMA): 8.6	µg L^{-1}	Speciation based analysis	Sakuma et al. (2010)
(tAs+MMA+DMA) >3.5	µg L^{-1}	Speciation based analysis	Fillol et al. (2010)
Urinary iAs as (AsIII + AsV+MMA+DMA): 9.1-1398	µg g^{-1}	Speciation based analysis	Valenzuela et al. (2007)
Females: 94.8 ± 250 Males: 59.7 ± 81.8	µg g^{-1} creatinine**	tAs	Sirot et al. (2009)
260	µg l^{-1}	tAs	Asante et al. (2008)
AsIII : <1-22.6 MMAV : <1-20.3 DMAV :17.7-86 AsV : <1-35.1	mg g^{-1} creatinine	Speciation based analysis	Agusa et al. (2006)
iAs: 1.1-1.6 iAs+MMA+DMA: 33.1-84.8	µg Ll^{-1}	Speciation based analysis	Hata et al. (2007)
tAs: 1000-6200 DMAV: 20-98 MMAV: 3-33 iAs: 1.2-62	µg L^{-1}	Speciation based analysis	Lindberg et al. (2006)
172	µg L^{-1}	tAs	Hall et al. (2006)
(tAs+MMA+DMA): 232-301	µg L^{-1}	tAs as sum of species	Concha et al. (2010)
11.1-54.5	µg g^{-1} of creatinine**	tAs	Maharjan et al. (2005)
AsIII + AsV : 7.1 DMAV : 41.7 MMAV : 5.6 tAs as sum of species: 47.9	µg L^{-1}	Speciation based analysis	Wilhelm et al. (2004)
10.1% of the human subjects found with highest bladder cancer risk calculated from Urinary arsenic and cumulative arsenic exposure	µg L^{-1}	Speciation based analysis	Chen et al. (2003)
IAs: 11-509.4 MMA: 55-2192.5 DMA:6.8-687.4	µg L^{-1}	Speciation based analysis	Loffredo et al. (2003)
2.48-4.05	µg g^{-1} creatinine**	tAs	Spěváčková et al. (2002)
2.2–106	µg L^{-1}	tAs	Matschullat (2000)
<0.1-18.32	µg L^{-1}	tAs	Gebel et al. (1998)

Reported levels	Unit	Arsenic testing as	References
30-2000	µg L^{1}	tAs	Das et al. (1995)
tAs: 13-440 iAs+MMA+DMA: 9-405	µg L^{-1}	Speciation based analysis	Vahter et al. (1995)
AsIII: 0.5-35 DMAV: 15-85 MMAV: 4-36 AsV: 3-57	µg L^{-1}	Speciation based analysis	Harrington et al. (1978)

*Units vary in accordance with testing methods
**Urinary arsenic reference value: 28 µg $mmol^{-1}$ creatinine

Despite many studies on urinary arsenic metabolites, it is still far from clear what the processes are that control the uptake and excretion of arsenic species from different dietary sources and how these different exposures lead to health impacts (Rivera-Nunez et al., 2012).

2.3.7 Arsenic Health Impacts

Chronic health problems result from prolonged exposure of humans to arsenic (Hong et al., 2014). Responses to arsenic exposure vary depending on genetics as much as exposure levels but it might be supposed that certain vulnerable groups, e.g. pregnant women, infants, children, the elderly, and immune-compromised groups are at greater risk of health impacts (European Food Safety Agency, 2009, Georgopoulos et al., 2008, Kordas et al., 2007). A number of epidemiological studies, from cohort to case-control, have evaluated the role of arsenic exposure for a number of health outcomes (Table 2.7).

The Health Effects of Arsenic Longitudinal Study (HEALS), the largest cohort study in the world, evaluated individual-level tAs exposure for 12,000 people in Araihazar, Bangladesh (Ahsan et al., 2006). HEALS indicated the prevalence of risk at levels below the current WHO and USEPA permissible limit for arsenic in drinking water, shown by 24% of the participants drinking water with arsenic less than 10 µg L-1. Biomarker samples of urine and blood were taken providing recent exposure data but chronic exposure proxies available via hair and nail samples were not evaluated. Whilst the study did model food intake, food samples were not collected and characterised, as dietary sources other than drinking water were considered negligible.

The results of epidemiological studies (Table 2.7) are further supplemented by toxicological studies, which used animal models to identify a link between

gastrointestinal problems and lung cancer due to arsenic exposure (Afolabi et al., 2015, Santra et al., 1999). As with all animal studies, caution is required when translating to humans particularly from rodent models (Tokar et al., 2010, International Agency for Research on Cancer, 2012b). In general, the health effects reported by most studies (Table 2.7) for various exposure levels were generally inferred on the basis of statistical correlation between tAs in drinking water, excreted urinary arsenic metabolites and existing physical symptoms (Chen et al., 2013, Agency for Toxic Substances and Disease Registry, 2007, Tsai et al., 1999). However, such analyses do not necessarily provide conclusive evidence of the role of individual arsenic species, particularly exposure over the long-term, in disease development. For instance, few studies have evaluated the toxicity of DMA (US Environmental Protection Agency, 1993) and MMA relative to AsIII (Petrick et al., 2000, Petrick et al., 2001) although a recent investigation by Huang et al. (2014) have concluded that MMAIII potentially aggravates arsenic-associated cardiovascular disorders.

Table 2.7: Summary of reported health effects of higher levels of arsenic

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
Skin	Hyperpigmentation, Hyperkeratosis and Skin tumours	<50-3400 µg L^{-1}	cross sectional population survey	7683	tAs in water, examination of skin lesions	Mazumder et al. (1998)
	Prominent transverse white lines in the fingernails and toenails called Mee's lines	1 g of sodium arsenite in an apparent suicide attempt.	case-control study	1 (20 years old man)	urinary arsenic, neurological examination	Fincher and Koerker (1987)
	Skin lesions	<100 µg L^{-1}	prospective cohort study 668 with skin lesions and 10051 without lesions	11746	examination of pre-malignant skin lesions	Argos et al. (2007)
	-do-	115-380 µg L^{-1}	case control study based on cross-sectional survey	415 (256 identified cases)	tAs in water, medical examination of skin lesions	Haque et al. (2003)
	-do-	<100 µg L^{-1}	prospective cohort study (based on individual-level exposure assessment)	11,746 (married men and women)	-do-	Ahsan et al. (2006)
	Skin cancer	<500 µg L^{-1}	retrospective cohort study	3,179	well-use histories, medical history on dermatological examinations	Lamm et al. (2006)
Gastrointestinal system	Diarrhoea and stomach issues	slow poisoning case with 36000 µg L^{-1} arsenic	case-control study	1(62-year-old man)	tAs, autopsy findings, post-mortem toxicological findings	Poklis and Saady (1990)

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
	Non-cirrhotic portal fibrosis	5050-14200 µg L^{-1}	hospital-based and case control cohort follow-up studies	248 patients	Liver function tests, HBsAg status. Liver biopsy	Santra et al. (1999)
	Macro-nodular cirrhosis variceal bleeding	0.015–0.06 mg kg^{-1} per day	clinical study (8 patients, who received arsenical preparation for psoriasis as Fowler's solution)	8	tAs, clinical examination	Nevens et al. (1991)
	Liver dysfunction Haemangio endothelioma	240-2000 µg L^{-1}	retrospective cohort study (16 male patients with malignant tumours associated with arsenic-polluted water)	16	tAs in water	Zaldívar et al. (1981)
Cardiovascular system	Cardiovascular disease	3 to 295 µg m^{-3}	retrospective cohort study (based on causes of death among a group of 527 pensioners in a copper smelter)	527	airborne arsenic, urinary arsenic values	Pinto et al. (1977)
	-do-	<0.5->0.5 mg m^{-3}	case-control retrospective assessment of exposure	325 (74 referents and 251 individuals)	airborne arsenic, in a Swedish copper smelter	Axelson et al. (1978)
	-do-	0.9-21.65 mg m^{-3}	case-control study (based on copper smelter employees in Montana)	8,045 (302 died with respiratory cancer)	estimated measures of relations between respiratory cancer mortality and exposure to airborne arsenic	Lee-Feldstein (1989)

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
	-do-	>40 µg L^{-1}	case control study	298 cases and 275 controls	total iAs in water and toenail samples (Nail arsenic above 1.38 µg g^{-1} concluded to be associated with an increased risk of cardiovascular disease)	Wade et al. (2015)
	-do-	≥ 108 µg L^{-1}	case–cohort prospective study	369 incident fatal and non-fatal cases of CVD	Blood pressure monitoring, verbal autopsy procedure, medical records, death certificates, determination of arsenobetaine (AsB), arsenocholine (AsC), AsV, AsIII, MMA, and DMA in urine samples.	Chen et al. (2013)
	-do-	exposed to 50, 100 and 150 mg L^{-1} arsenic)	clinical study	based on male albino rats	induced lipotoxic and non-lipotoxic dyslipidemia at “low” or “medium” doses,	Afolabi et al. (2015)

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
	Hypertensive heart disease	14 to 166 µg L^{-1}	cohort mortality study (association of drinking water arsenic and mortality outcome)	2,203 deceased cases	tAs in water	Lewis et al. (1999)
	Hypertension		case control study	40 (workers occupationally exposed to arsenic)	tAs in urine samples, determination of glycosylated haemoglobin (Hgb A1C)	Jensen and Hansen (1998)
	Ischaemic heart disease	267.05 ± 20.95 µg L^{-1}	cross sectional study	1081	Mean tAs of water 267.05 µg L^{-1}, urinary iAs and its metabolites	Huang et al. (1998)
	Cardiac arrhythmias		patient based case control study	1(57-year-old man)		Goldsmith and From (1980)
	Peripheral vascular disease	80 µg L^{-1}	cohort (follow-up)	774 (129 adults, 645 school children)	tAs content in hair and nail clippings, vegetables and beverages samples, examination of cutaneous lesions attributed to arsenicism	Borgoño et al. (1977)
	Peripheral vascular disturbances leading to gangrene, and; Black foot disease	>10 µg L^{-1}	cohort (follow-up) study	survey of 40,421 inhabitants and follow-up of 1,108 patients	tAs in water, examination of skin lesions, calculation of death rates specific for age for black foot disease	Tseng (1977)

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
Respiratory diseases	Restrictive or obstructive Lungs diseases, and bronchitis	0.015–0.08 mg kg^{-1} per day	cross-sectional survey	7683	tAs in drinking water, chest X-ray and HRCT	Mazumder et al. (1998) Mazumder et al. (2000)
	Lungs diseases	780 µg L^{-1}	cohort (follow-up) study	20067	death certificates from Black Foot Endemic area of Taiwan from 1971 to 1994)	Tsai et al. (1999)
	-do-	Mean 800 µg L^{-1}	cohort (follow-up)	774 (129 adults, 645 school children)	tAs content in hair and nail clipping, vegetables and beverages samples, examination of cutaneous lesions attributed to arsenicism	Borgoño et al. (1977)
	-do-	>250 µg L^{-1}	population-based prospective cohort study	20,033 adults	tAs in drinking water (tube-well), urine and blood samples, collection of arsenic exposure history, smoking and demographic data, Pulmonary function test	Parvez et al. (2013)
	Lung cancer	10- 1752 µg L^{-1}	cohort (follow-up) study	308 lungs cancer cases	death certificates of residents who died from cancers during the period from 1973 to 1986	Chen et al. (2010c)
Endocrinology	Diabetes mellitus	0.11 mg kg^{-1} per day	case control study	40 (workers occupationally exposed to arsenic)	tAs concentration in urine samples, concentration of glycosylated haemoglobin (Hgb A1C) in 40 arsenic workers.	Jensen and Hansen (1998)

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
	-do-	500-1000 µg L^{-1}	case–control (case–comparison)	163 exposed subjects and 854 unexposed individuals	tAs in water samples, history of symptoms, previously diagnosed diabetes, determination of glucosuria, and blood sugar level after glucose intake.	Rahman et al. (1998)
Neurological disorders	Peripheral neuropathy, and Hearing defects	0.005–0.11 mg kg^{-1} per day	case control study (neurological effects)	56 (10-year-old children residing near a power plant burning local coal of high arsenic content).	audiometric and clinical examination	Bencko et al. (1977)
	Cerebrovascular disease 10–100 µg/L	10–100 µg L^{-1}	ecological study (based on standardized mortality ratio (SMR) analysis	8593 observations for cerebrovascular diseases	tAs in 9251 well water, Michigan resident death files data for 1979- 1997	Meliker et al. (2007)
Haematopoietic system	Disturbed erythropoiesis with anaemia	chronic arsenic intoxication	case report study	1 (47 years patient exposure to a weed spray approximately 2 weeks prior to admission).	arsenic contents of tissues, clinical examination of patient, bone marrow examinations	Westhoff et al. (1975)
Reproductive system	Increased frequency of miscarriages	6-978 µg L^{-1}	prospective cohort study	1,578 mother-infant pairs	tAs in urine collected at around gestational weeks 8 and 30	Rahman et al. (2008)
	Foetal losses	174-319 µg L^{-1}	spatiotemporal analytical study	26,972 pregnancies	spatiotemporal analysis, spatial scan test used to identify unique non-	Sohel et al. (2010)

Organs targeted	Health impacts	Arsenic exposure level	Study type	Participants No.	Parameters studied	References
					random spatial and spatiotemporal clusters of foetal loss and infant deaths	
Genitourinary system	Nephritis and prostate cancer	53-750 µg L^{-1}	cohort (follow-up)	2,203 deceased cases	nephritis (SMR = 1.72; CI, 1.13-2.50), prostate cancer (SMR = 1.45; CI, 1.07-1.91)	Lewis et al. (1999)
	Bladder cancer	18-164 µg L^{-1}	cohort (follow-up) study	312	death certificates from Black Foot Endemic area of Taiwan	Tsai et al. (1999)
	-do-	170-800 µg L^{-1}	ecological study (based on the dose-response relationships between cancer risks and the concentration of iAs)		risk estimate of 1/1000 persons	Smith et al. (1992)
	Kidney cancer	60- 860 µg L^{-1}	case-control study	122 kidney cancer cases and 640 population-based controls	tAs in water, water consumptions with individual data on exposure and potential confounders during 2007–2010)	Ferreccio et al. (2013)

mg kg^{-1} is equivalent to 1000 µg kg^{-1}
mg m^{-3} is equivalent to 1 µg L^{-1}

2.3.8 Arsenic permissible limits for water and food

The WHO international standards for drinking water established a maximum acceptable level of 50 µg L^{-1} in 1963 for tAs in drinking water (World Health Organization, 2008). This limit was reduced to 10 µg L^{-1} in 1993, based on concern regarding its carcinogenicity (World Health Organization, 2008, Smith and Smith, 2004). This lower guideline value has been adopted by many statutory bodies in industrialized nations, including the United States (U.S Environmental Protection Agency), Canada (Health Canada), and the European Union. However, many developing countries have generally kept the higher level of 50 µg L^{-1}. As such, millions of people in several developing countries (Bangladesh, China, India, Nepal, Thailand, Vietnam, Pakistan; Cambodia, Myanmar, Iran, Ghana, Argentina, Croatia) are still using drinking water with arsenic above 10 µg L^{-1} despite evidence of a carcinogenic effect (The World Bank, 2005). The level of arsenic in drinking water below which no health effects can be observed, or the highest sensitive toxicity end-point, below which there is no risk of carcinogenicity, is yet to be confirmed. Following this, the limits of 10 and 50 µg L^{-1} apply to iAs only and do not consider the varying toxicity of different arsenic species – from highly toxic AsV to less toxic organic species.

The WHO guideline limits only apply to water sources: exposure to arsenic–contaminated foodstuffs has only been considered by two national governments. Australia has established a limit of 1 mg kg^{-1} and China set a limit range of 0.05-1.5 mg kg^{-1} for vegetables, fruits, eggs, milk, rice, flour, beans/pulses fish and sea foods (Das et al., 2004, Islam et al., 2004, Jahiruddin et al., 2004, Abedin et al., 2002, Japan International Cooperation Agency/Asia Arsenic Network, 2004). Furthermore, the Current Codex Alimentarius, or 'food code', sets a maximum limit of 0.2 mg kg^{-1} of arsenic in white rice and 0.4 mg kg^{-1} for brown rice (Codex Alimentarius Commission, 2014). The development of limits imposed on foodstuffs demonstrates growing concern regarding arsenic availability in food and has important implications for food exports. As for water, the limits are based on tAs rather than individual arsenic species.

2.3.9 Risk assessment of arsenic species

Risk assessment tools identify likely health outcomes resulting from exposure to hazards and therefore are crucial first steps in determining the need for the development of risk management strategies and/or the need for regulation. A range of different risk assessment techniques, approaches or models (Table 2.8) have been used for arsenic (Chen et al., 2010b, Mondal et al., 2010, Mondal et al., 2008, Ling et al., 2005, Liao et al., 2008).

Input variables for these methods have generally included estimates or measured concentrations of tAs in water; fewer studies have included a food source variable and these tend to have a restricted sample size or do not integrate the different exposure sources (Mondal et al., 2010, Saipan and Ruangwises, 2009). Similarly, few studies considered the risks posed by individual arsenic species specifically, trivalent (AsIII, MMAIII and DMAIII) or pentavalent species (AsV, MMAV and DMAV) from different exposure sources: the few studies that do this tend to use predicted arsenic species calculated from tAs levels and focus on an ecological, rather than a human health risk assessment (Markley and Herbert, 2009, Du et al., 2015). For human health risk assessment, arsenic speciation and bioavailability are critical as arsenic species vary differ in their toxicity and bioavailability and thus influence the uptake dose resulting from dietary intake (Laparra et al., 2005). It is thus important to obtain information about the arsenic species absorbed from food, water, and soil, metabolized in the liver and kidneys, accumulated in nails and hair, and ultimately eliminated by urine and faeces.

Table 2.8: Summary results of methodologies and tools adopted for risk assessment

Technique/ Tool Used	Location	Exposure sources	Risks assessed for form of arsenic	Risk output	Reference
Species sensitivity distribution (SSD) and assessment factor (AF) methods for ecological risks	China	River water and sediments	AsV, AsIII, MMA and DMA	Ecological risk from AsIII and AsV <1	Du et al. (2015)
Summary Relative Risk Estimate (SRRE)	Taiwan (Southwest)	water	tAs	Non-significant (SRREs <1.0) results at low dose *vs*. predicted risk using high-dose extrapolation	Tsuji et al. (2014)
Log-Logistic model	USA	apple juice	tAs	Total cancer rate (per million) at ≥10 µg L^{-1}: 8.0 (0.0, 21.3)	Carrington et al. (2013)
Mantel-Cox Method	Taiwan (Northeastern Coast)	water	tAs	Hazard ratio ranged from 1.0-8.71 for urothelial carcinoma by arsenic exposure at <10-100 µg L^{-1}	Yang et al. (2013)
Generalized estimating equation (GEE) models	Bangladesh	water	tAs	Every log_{10} decrease in water and toenail arsenic was associated with 22% relative increase in skin lesion recovery	Seow et al. (2012)
Biologically-Based Dose–Response (BBDR) Model	USA	Comparative genomic data from individuals with known exposure from drinking water	iAs	*in vitro* dose response is nonlinear for urinary cancer	Clewell et al. (2007)
USEPA one-hit model (1989)	West Bengal, India	water rice	tAs	Median excess lifetime cancer risk above USEPA regulatory threshold target cancer risk level of 10^{-4}–10^{-6}	Mondal et al. (2010)

Technique/ Tool Used	Location	Exposure sources	Risks assessed for form of arsenic	Risk output	Reference
USEPA Risk Assessment Approach	Pakistan (Kohistan region, northern areas)	water	tAs	Low chronic risk with HQ >1 (Jabba, Dubair) and medium cancer risk with HQ <1	Muhammad et al. (2010)
	Vietnam (Four villages in Ha Nam province)	water	tAs	Potential carcinogenic rate of 5 in 1000 people	Nguyen et al. (2009)
	Thailand (Ronphibun)	Water, food	tAs	HQ = 6.98 CR = 1.26 x 10^{-3}	Saipan and Ruangwises (2009)
	Turkey (Izmir)	water	tAs	HQ: 41 in 19% of the population Carcinogenic risk of < 10^{-4} in 46% of population Carcinogenic risk >10^{-6} in 90% of population	Kavcar et al. (2009)
	USA	water	AsV, AsIII, or DMAV (without model validation)	Groundwater: minimal chronic exposure risk (< 10^{-6}) by DMAV Surface water: lifetime cancer risk (>10^{-4}) of AsIII	Markley and Herbert (2009)
Cox's Proportional Hazards Regression Models	Taiwan (North-eastern Coast)	water	tAs	significant dose–response trend (P= 0.001) of lung cancer risk	Chen et al. (2010b)
Integration of Weibull dose–response function and a physiologically based pharmacokinetic (PBPK) model	Taiwan (Southwestern and northeastern Taiwan)	water	tAs	Positive relationships between arsenic exposures and cumulative incidence ratios of bladder, lung, and urinary-related cancers i.e. r^2 = 0.58–0.89.	Liao et al. (2009)
NRC multistage Weibull model	Taiwan vs Chakdha block, West Bengal	water	tAs	Death and DALYs calculations are sensitive to the choice of dose–response model	Mondal and Polya (2008)

Technique/ Tool Used	Location	Exposure sources	Risks assessed for form of arsenic	Risk output	Reference
Cumulative Arsenic exposure Index''(CAI)	Bangladesh	water	tAs	CAI of 1.64–49341.62 mg with arsenic exposure of 0.1–864 mg l^{-1}	Ahsan et al. (2006)
Physiologically Based Toxicokinetic & Toxicodynamic (PBTK/TD) Modeling	Taiwan (Southwestern)	Tilapia farm fish	tAs	All predicted 90th percentiles of HQ<1 for city residents and subsistence fishers in the BFD area, indicating small contributions from farmed tilapia consumption	Ling et al. (2005)
Death and Disability Adjusted Life Years (DALYs).	Bangladesh	water	tAs	7930 YLDs lost due to arsenicosis, which accounts for 1908 DALYs	Molla et al. (2004)
Monte Carlo modelling	USA	Water, air, soil, food	iAs	Food is more significant for arsenic exposure than water	Meacher et al. (2002)

2.4 Synthesis

There have been many studies evaluating the distribution of tAs in water, food, soil and human biomarkers but relatively few have included arsenic species characterisation (Tables 2.1-2.6). Understanding the contribution of individual arsenic sources to overall arsenic burden is important in developing the most appropriate risk mitigation strategies. Understanding the burden of each arsenic species and the interaction of species from source though intake and uptake to accumulation/metabolism and toxic effect is also a pressing need. Current literature provides good information on pathways from some sources, in particular drinking water, to health outcomes but the underlying biological mechanisms affecting the uptake and metabolism of different arsenic species from a range of sources are still not well understood.

As previously mentioned, linking environmental concentrations of arsenic to the levels identified in biomarker analyses have been carried out by relatively few studies. Comparing studies of similar geographical origin reported in Tables 2.1-2.6, Pearson's correlation analyses were undertaken as part of this review to examine relationships between tAs levels in water, soil, food and humans (as biomarkers) to help understanding of pathways of exposure and uptake. Positive and significant correlations were found between arsenic in soil and water ($r=0.830$, $p=0.000$, $n=20$), arsenic in water and hair ($r=0.563$, $p=0.029$, $n=15$), water and urine ($r=0.687$, $p=0.005$, $n=15$), hair and nail ($r=0.829$, $p=0.011$, $n=8$), and nail and urine ($r=0.925$, $p=0.024$, $n=5$). The linear correlations suggest that elevated levels of arsenic in the biomarkers are most likely a consequence of the intake of arsenic-contaminated water. The close correlation of the three biomarkers also demonstrates that they are inter-related.

Many of the models used to predict carcinogenic or non-carcinogenic health outcomes from arsenic exposure require data specific to an exposure scenario that might not always be available to the assessors. Hence, the use of generic exposure data, such as that available through the USEPA Exposure Factors Handbook (United States Environmental Protection Agency, 2011) and the EFSA Comprehensive European Food Consumption Database (European Food Safety Agency, 2011), are often used and whilst a good surrogate where no data exist,

this does lead to assumptions about consumption patterns and concentrations (e.g. tAs but not individual arsenic species).

Providing an integrated approach to arsenic risk assessment is likely to have been prevented by a number of factors including lack of speciation facilities, high cost of arsenic speciation, uncertainty levels of speciation modelling, and physiological differences of humans and animals for toxicological assessment. Nevertheless, such an approach would consider all possible exposure sources, ingestion pathways, response elements, and health outcomes, and include the contribution made by individual arsenic species to each step.

2.5 Conclusions and research needs

Arsenic in water, food, soil and human biomarkers exists at various concentrations and in different chemical forms (AsIII, AsV, MMAIII, DMAIII, MMAV and DMAV). Arsenic released from natural geological, anthropogenic or multiple sources enters groundwater and soil with levels reported up to 24000 µg L^{-1} and 43,500 mg kg^{-1} respectively for water and soil. Uptake by plants from soil or water has led to arsenic residues identified in many vegetable and cereal crops as well as fish and seafood, where it accumulates in the food chain. As such, different dietary sources including drinking water contribute to arsenic intake. Biomarker assessment in humans further demonstrates bioaccumulation, metabolism and excretion. Most studies evaluating human exposure to arsenic have concentrated on tAs; relatively few have looked at the role of individual arsenic species and this is a pressing research need. Furthermore, integrated approaches to exposure and thereafter risk assessment that consider all sources of arsenic exposure are not commonly reported, despite arsenic sources and exposure being relatively well studied. Nevertheless, the risks of arsenic exposure, both carcinogenic and non-carcinogenic, are well-reported and demonstrate the importance of developing risk assessment approaches that can fully elucidate the different sources of exposure and hence suggest appropriate mitigation and management steps to reduce exposure.

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Chapter 3: General Methodology

3.1 Risk Assessment Guidelines and Frameworks

The available guidelines and frameworks for risk assessment include US Environmental Protection Agency (USEPA), Agency for Toxic Substances and Disease Registry (ATSDR), and Australian e-Health Environmental Health Risk Assessment framework (2012). Furthermore, Human health risk assessment of priority substances (Health Canada, 2008), European Chemical Bureau's Regulation (EC No 1488/94) on Risk Assessment for existing substances (European Commission, 2003) and European Food Safety Authority (EFSA Scientific Committee, 2010). Most of these frameworks, although including some differences, use a similar tier based approach (Figure 3.1) depending on the objectives, data availability and resources.

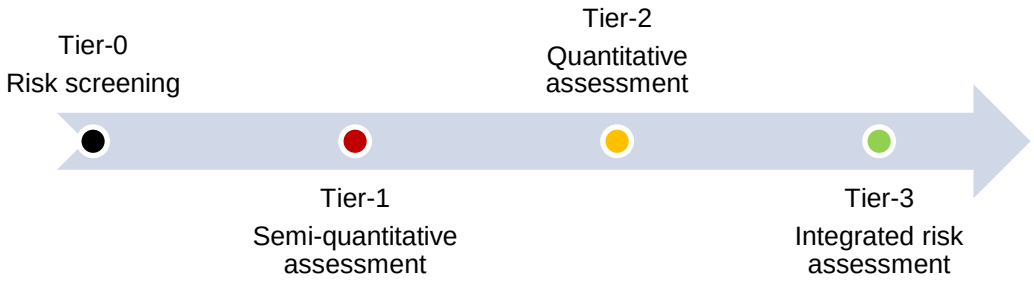

Figure 3.1 Tier based approaches of risk assessment

The key tasks of each tier include problem identification, receptor characterisation, exposure assessment, toxicity assessment, and risk characterisation in order to make a risk management decision. The risk assessment design is typically based on questionnaires, records, laboratory tests, physical measurements and other specific procedures (Kelsey, 1996). In this study, the receptor based risk assessment is conducted using a direct approach (bio monitoring) and indirect approach (exposure modelling). The advantages of bio monitoring are reduction in analysis errors in exposure assessment exercises which sometimes recall for repeated exposure measurements (World Health Organization, 2015). Epidemiological research to evaluate the relationship between dietary intake and disease susceptibility

requires dietary intake data. Structured FFQs are generally based on the list of foods, frequency of consumption, portion size consumed and number of days for dietary data recording (Franco et al., 2016; Coulston et al., 2013). To ensure in-time recording, self-reporting participants are advised to record their food types, total number of servings and serving size for each food type, including drinking water at the eating occasion (Cade et al., 2002) or at the end of each day (Kurzius-Spencer, 2012). Compared to structured FFQs, technology aided new methods are expensive and challenging for less literate populations (Shim et al., 2014). The socio-economic background of a study population may limit the methods use in some population groups (e.g. low literacy, children and elderly groups). In such cases, investigators or interviewers also help the study participants in data entries.

3.2 Methods applied to conduct integrated risk assessment

The generic methodology adopted to address each of the study objectives is schematized as Figure 3.2 and summarized in the following section. Specific methods are given in the respective chapters.

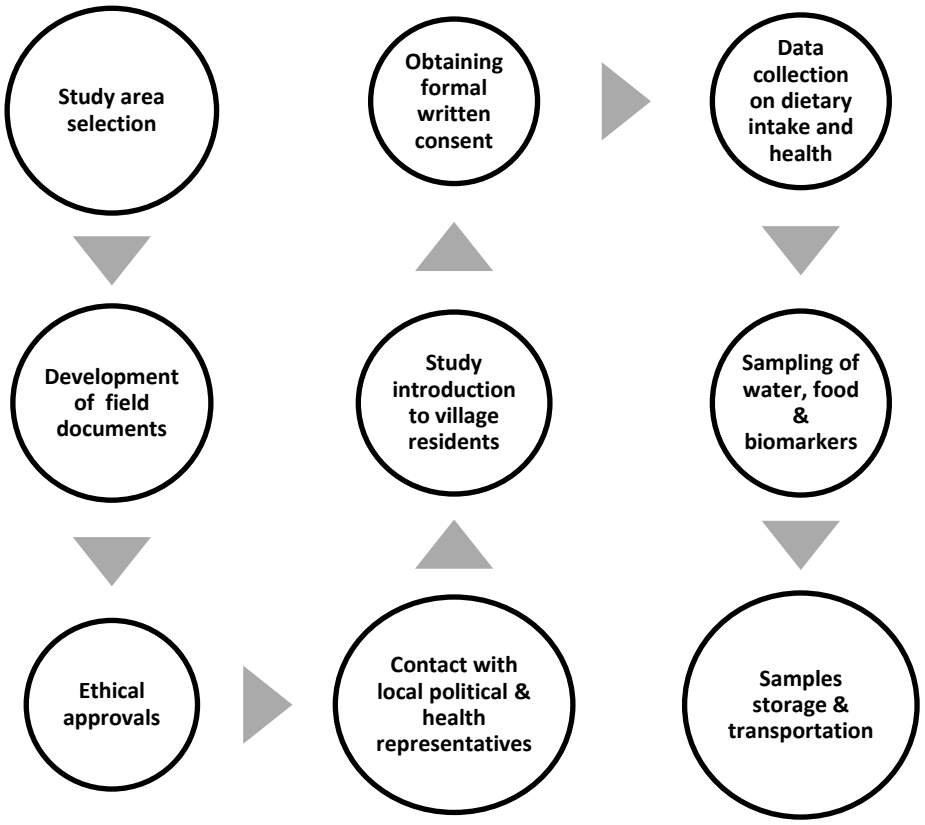

Figure 3.2: Sequence of actions performed in field

3.1 Study area selection

The Indo-Gangetic basin consists of Bangladesh, India, Pakistan and southern Nepal, with a population above 750 million and responsible for 25% of global groundwater abstraction (MacDonald et al., 2016). The geological conditions of this region associated with arsenic-laden sediment of the Himalayas have given impetus to explore the intensity of arsenic induced risks in Pakistan. In Pakistan, the concentration of arsenic in groundwater of some districts of Punjab and Sindh provinces has been observed through different studies (Tahir and Raheed, 2014; Ahmed et al., 2004). Four districts (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan (RYK) in Punjab province were selected based on a previous study (Ahmed et al., 2004). The locality chosen for the study corresponds to distant rural settings in these four districts, where arsenic assessment and speciation study had not been conducted previously. This included six villages identified to have at least one groundwater source with levels of arsenic in excess of 50 µg L^{-1} in a preliminary survey and were selected as the study sites. The study villages such as Badarpur (district Kasur)**,** Chak-46/12-L, Chak-48/12-I, Chak 49/12-I (Sahiwal district) and Basti Balochan (district Bahawalpur) are located between the Sutlej and Ravi rivers, whilst Basti Kotla Arab (District Rahim Yar Khan) is located in the alluvium plain between the Cholistan desert in the east and Indus River in the west. Ground water (tube wells, dug wells and hand pumps) is the major water source in all the four districts. Water pollution from raw sewage, industrial wastes, and agricultural runoff, and limited natural fresh water resources were reported to be the major environmental threats (Government of Pakistan, 2009). The climate of the study areas is extremely hot, reaching 45-50 ^{o}C in summer and is cold and dry in winter; down to 5-10 ^{o}C (Government of Pakistan, 2009). Most of the study villages were occupied by crop fields and the main crops were wheat, cotton and sugar cane, thus farming was found to be the main occupation of the residents. An overall socio-economic status of the study area residents is poor.

3.2 Sampling frame for cohort enrolment from the study area

A sample size of 398 from 223 households was recruited to the project, derived from a formula (Equation. 3.1) for estimating sample proportions from large

populations (Collett, 2003). A 95% confidence level and standard error of 0.05, as recommended by (Collett, 2003), assumes a statistically significant sample size of 384 respondents for a large population. Additional volunteers (n=14) were also included in the study as alternatives in case of participant's withdrawal from the study at later stages.

$$n = \frac{t^2 \times \rho\,(1-\rho)}{m^2} \qquad \textit{(Eq.3.1)}$$

n = estimated sample size
t= the critical value obtained from a standard normal distribution. For each level of confidence there is a corresponding value of z. (95%: corresponding z value of 1.96)
p= estimated prevalence of arsenic contamination in the study area (prevalence of 0.5)
m=margin of error at 5% (standard value of 0.05).

The total number of households in the six selected villages was 1776 as provided by Pakistan Bureau of Statistics (2014) on demand. The resultant sampling fraction for this study came out to be 12%. Following the sample size, the cohort of 398 participants was selected randomly to ensure that the study would provide a true exposure scenario of the population in six villages. Selection of study participants was based on the criteria that they have lived in their villages at the time of the study for the last 5 years and children (<5 years) by birth, consuming water from their household hand pump or well, non-smoking and have submitted the informed consents. Efforts were made to have maximum participation from members of the same house to assess inter-individual variability.

3.3 Ethical approval and field questionnaires

The research protocol of this study was approved by the University of Leeds Research Ethics Committee and National Bioethics Committee of Pakistan (Appendices 3.1 and 3.2). A set of field information sheets, questionnaires and sampling proformas were developed in English (Appendices 3.3 and 3.4) and Urdu languages and used for introducing participants with the aims of the study, seeking their consent for participation in the study (Appendix 3.5), data collection on demographic features, 24-hours water and food intake diary (Appendix 4.1), physical examination (Appendix 8.1), water, food and biomarkers sampling.

3.4 Field survey

The five membered field team, led by the author, consisted of three research associates (two females and one male) hired from the National Water Quality Laboratory of Pakistan, one registered and trained health worker and one registered physician. Field team members were fluent in Urdu, Punjabi (local language) and English, and well aware of local culture and customs. Training was imparted to the team members on administration of field documents, informed consent procedures, data and sample collection.

This was the first study of its kind in the study area and based on the local socio-cultural conditions communication tools such as mosque announcements, local political representatives and distribution of informative leaflets through basic health units (BHUs) were used to ensure maximum participation of the local residents in the study.

During an initial visit to each study village, residents were briefed on the study rationale and objectives and their expected participation. As a result, 398 eligible individuals were identified to be potential participants who were enrolled into the study cohort and interviewed by the field team. Participants were given the option to consent with or without providing the water and food intake data, providing their biomarker samples or photographs reflecting skin disorders. The information on the option for withdrawal from the study at any stage was also provided to the enrolled participants. An appointment was made during July-September, 2014 for the field team to visit households for administration of the water and food frequency questionnaire, water, food and biomarkers sampling as well as for examinations of arsenical skin lesions. Initial visits to the six villages prior to the project start had identified the following challenges:

a) People with rural backgrounds having lower literacy rates and language barriers.
b) Extreme hot weather and severe power crisis during field work, a possible constraint to preserve, store and transport cooked rice and urine samples at -20 ^{o}C and water samples below 4 ^{o}C.
c) Social issues among rural residents due to poverty, arsenic induced skin problems and gender discrimination resulted in lack of cooperation from rural communities with field team.
d) Limited availability of men in day time due to working in crop fields.

Accordingly, the field work strategies and schedules were prepared to meet these challenges. They included; inclusion of female team members, awareness of cultural and social norms and ability to communicate with householders in the most appropriate local language, arrangement of uninterruptible power supply (UPS) and power batteries to supply continuous power to a field refrigerator and freezer for controlled samples storage. To reduce the field team and respondent bias, water sampling was undertaken as a parallel activity and results were produced after survey completion.

3.4.1 Dietary intake record

A method similar to the water diary method (Watanabe et al., 2004; Ohno et al., 2007) was used for recording daily water intake. A semi-quantitative FFQ was designed based on the dietary culture of the study area and this was organized according to food hours (i.e. morning, noon, afternoon, evening and night). Participants were instructed to fill-in the food type, number of servings consumed and its preparation source i.e. house, restaurant etc. Each family was given measurement aids to estimate the amounts of different foods and beverages consumed. Considering the low literacy rates of study area residents (44% without any formal education), field team interviewers completed the FFQ through in-person interviews. Total water intake and total intake of each food item was determined for each study participant. More specific methodology is given in Chapter-4.

3.4.2 Identification of skin manifestation

The prevalence of arsenic related skin manifestations had not been systematically studied in the study population and was evaluated as a biological marker of individual exposure. Health care services in these rural settings were not well organized to hold systematic patient's records to track their medical history. Therefore, study participants were observed and interviewed at their houses initially by the trained health worker, to record observations on the presence or absence of skin lesions in a structured questionnaire (Appendix 8.1). Study participants were finally screened for skin lesions at the basic health unit (BHU) of each village by the team physician having dermatological expertise and aware of diagnostic guidelines (Figure 3.3) of UNICEF clinical diagnostic manual

(Sun Guifan et al., 2004). A digital camera was used for taking photographs of skin manifestations of willing participants who have consented for their picture without facial identification. More specific methodology is given in Chapter 8.

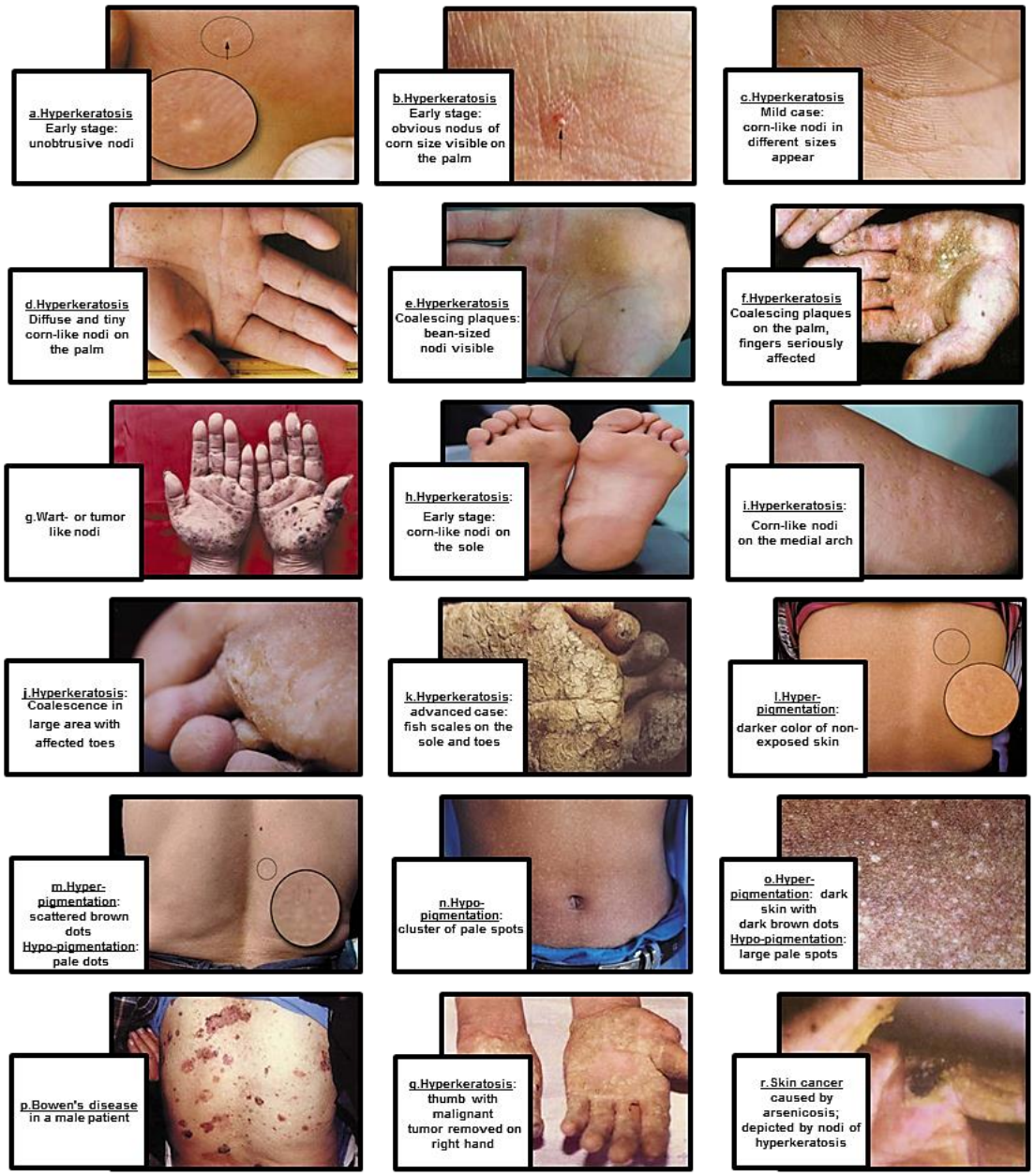

Figure 3.3: Diagnostic key of mild to advanced stages of arsenicosis
(a-k): Early, mild and advance stages of hyperkeratosis on palm and soles; figures (l-p): early and mild symptoms of hyper pigmentation and hypo pigmentation; figures (q-r): Advance and carcinogenic complications. *Source: UNICEF clinical diagnostic manual (Sun et al., 2004)*

3.4.3 Sampling

Concurrent with the dietary assessment survey, samples of household ground water, staple food (raw and cooked rice, wheat) from study households and

biomarkers (hair, toenail and urine) from study participants were obtained. Three additional households were willing to provide their ground water samples, whereas three of the study participants were not willing to submit their biomarker samples. In total, 228 water samples and 395 biomarkers samples were obtained. A Geographical Positioning System (GPS) was used to record coordinates (latitude and longitude) of each household water source in the sampling profile. Depth of ground water source and an estimated date of source development was also obtained. All the samples were collected in respective coded containers and preserved and stored before being transported under controlled conditions and processed for testing of tAs, inorganic and organic arsenic species.

3.5 Laboratory testing

Samples analysis for total arsenic and As species required pre-treatment, extraction and sample storage under a controlled environment. Considering these fundamental requirements, high purity chemicals and reagents were used for analysis of tAs and arsenic speciation. All pyrex and plastic ware used for analytical work was cleaned prior to use by soaking in 5% nitric acid overnight, rinsing with double distilled water (DDW) and storing clean. Using USEPA method 200.8-modified (US Environmental Protection Agency, 1994) and USEPA method 3050b (United States Environmental Protection Agency, 1996), samples were mainly processed and analysed on inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) and ion chromatography inductively coupled plasma collision reaction cell mass spectrometry (IC-ICP-CRC-MS). The quality of analytical work was checked by the analysis of NIST (National Institute of Standards and Technology) traceable standard reference materials, blanks, duplicates and spikes. Sample specific processing, analytical and quality control methodologies are given in subsequent chapters on water (Chapter 5), food (Chapter 6) and biomarkers (Chapters 7 and 8).

3.6 Exposure and risk assessment

Exposure and human health risks for cancer or non-cancer risk of As and its species (AsIII, AsV, DMA, MMA) were assessed for each arsenic species and for

each category of samples (water, rice and wheat) separately adopting deterministic (point estimation) and probabilistic risk assessment modelling approaches. These included USEPA Guidelines for exposure assessment (US Environmental Protection Agency, 1992), A Framework for Assessing Health Risk of Environmental Exposures to Children (U.S. Environmental Protection Agency, 2006), Process for Conducting Probabilistic Risk Assessment (US Environmental Protection Agency, 2001), and Dose-Response Assessments (US Environmental Protection Agency, 2010). A point estimate is a single numeric calculation of risk from chemical substances, whereas a probabilistic risk assessment (PRA) approach is used to analyse exposure data and risk quantification described as a distribution. PRA methods attempt to evaluate overall variability in the data and help to increase the accuracy by combining exposure levels across different pathways to produce the output risks as a distribution rather than point estimate. An elaborated methodology is given in Chapters 4, 5, 6 and 9.

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Chapter 4: Refinement of arsenic attributable health risks in rural Pakistan using population specific dietary intake values

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Abstract

Previous risk assessment studies have often utilised generic consumption or intake values when evaluating ingestion exposure pathways. If these values do not accurately reflect the country or scenario in question, the resulting risk assessment will not provide a meaningful representation of cancer risks in that particular country/scenario. This study sought to determine water and food intake parameters for one region in South Asia, rural Pakistan, and assess the role population specific intake parameters play in cancer risk assessment. A questionnaire was developed to collect data on sociodemographic features and 24-hour water and food consumption patterns from a rural community. The impact of dietary differences on cancer susceptibility linked to arsenic exposure was evaluated by calculating cancer risks using the data collected in the current study against standard water and food intake levels for the USA, Europe and Asia. A probabilistic cancer risk was performed for each set of intake values of this study. Average daily total water intake based on drinking direct plain water and indirect water from food and beverages was found to be 3.5 L day^{-1} (95% CI: 3.38, 3.57) exceeding the US Environmental Protection Agency's default (2.5 L day^{-1}) and World Health Organization's recommended intake value (2 L day^{-1}). Average daily rice intake (469 g day^{-1}) was found to be lower than in India and Bangladesh whereas wheat intake by adults (402 g day^{-1}) was higher than intake reported for USA, Europe and Asian sub-regions. Consequently, arsenic-associated cumulative cancer risks determined for daily water intake was found to be 17 in children of 3-6 years (95% CI: 0.0014, 0.0017), 14 in children of age 6-16 years (95% CI: 0.001, 0.0011) and 6 in adults of 16-67 years (95% CI: 0.0006, 0.0006) in a population size of 10000. This is higher than the risks estimated using the

US Environmental Protection Agency and World Health Organization's default recommended water intake levels. Rice intake data showed early life cumulative cancer risks of 15 in 10000 for children of 3-6 years (95% CI: 0.0012, 0.0015), 14 in children of 6-16 years (95% CI: 0.0011, 0.0014) and later life risk of 8 in adults (95% CI: 0.0008, 0.0008) in a population of 10000. This is lower than cancer risks in countries with higher rice intake and elevated arsenic levels (Bangladesh and India). Cumulative cancer risk from arsenic exposure showed the relative risk contribution from total water to be 51%, from rice to be 44% and wheat intake 5%. The study demonstrates the need to use population specific dietary information for risk assessment and risk management studies. Probabilistic risk assessment concluded the importance of dietary intake in estimating cancer risk, along with arsenic concentrations in water or food and age of exposed rural population.

4.1 Introduction

Diet has been suggested to be the key causal factor for approximately 30% of cancers in industrialized countries (Doll and Peto, 1996) and about 20% in developing countries (Willett, 1995). However, water and food consumption patterns differ across the different regions of the world and can even vary within a country due to diverse socio-economic situations, dietary/cultural preferences, ethnicity, climatic conditions, age and sex (World Health Organization, 2011). As such, careful consideration must be made when performing risk assessments of the intake patterns appropriate to the country/region or population for which cancer risks are being assessed.

In South Asia, there has been limited research into the association between diet and carcinogenic potential (Ganguli et al., 2011). Most such studies use data from epidemiological studies conducted in developed countries where diets and consumption patterns are usually very different. As an example, water consumption in South Asia might be considerably higher than the commonly used default water intake value of 2.5 L day^{-1} (United States Environmental Protection Agency, 2011) and 2 L day^{-1} for an adult (World Health Organization, 2011, European Food Safety Agency and Allergies, 2010) leading to an under estimate of exposure risk from waterborne chemicals such as arsenic. Similarly, rice

consumption in South Asia is generally considerably higher than in many developed countries (Food and Agriculture Organization, 1998) but even within South Asia, there will be considerable variation with large areas of India consuming half the rice per capita of Bangladesh but higher levels of wheat (National Statistical Organisation India, 2012, Meharg and Zhao, 2012).

Variations in dietary consumption patterns between different subpopulations in the region were rarely considered. For instance, information on age or gender specific dietary differences can be used to define subgroups at highest risk (Zahm and Fraumeni, 1995). Children can have higher exposures to dietary chemicals than adults probably due to higher ratios of food consumption per kg body weight resulting in higher relative daily doses (Moy and Vannoort, 2013). A study by the National Research Council (2013) found that children were at greater risk from ingestion of pesticide residues whilst a study by He et al. (2013) reported higher dietary cadmium exposure in men compared to women due to different consumption patterns of cadmium-containing foods such as cereals.

At a more local level, diets in urban areas are often very different to rural areas (Miller et al., 2012) for instance, in Pakistan, there has been an emphasis on metabolic and cardiovascular health risks from diet in urban areas that are not necessarily transferrable to rural areas with different social, cultural, economic and environmental factors affecting diets (Yakub et al., 2010; Hydrie et al., 2010; Jafar et al., 2009; Iqbal et al., 2004).

Dietary intake data must consider all potential dietary sources. However in the case of chemical risk assessment, some sources, particularly the contributions of indirect water intake and food, were often not adequately taken into consideration for consumption and associated risk assessment. Direct water is defined as tap water consumed directly as plain drinking water, whereas, indirect water is defined as water added to foods and beverages (e.g. tea, coffee, bottled water etc.) during final preparation at home or by food service establishments. Total water refers to combined direct and indirect water consumption (Bennett and Stedge, 2000).

This study sought to gather food and water intake data from rural villages in Pakistan to examine the influence of regional rather than generic intake estimates on human health risk assessments, specifically for cancer risk. It focuses on the

need to evaluate all key ingestion pathways including indirect water consumption, food intake and the role of socio-demographic factors such as sex, age and occupation on consumption patterns. A case study is provided based on arsenic exposure through ingestion of arsenic-contaminated water and food.

4.2 Materials and Methods

4.2.1 Dietary Intake methodology

Six villages in four districts (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan) of Pakistan were identified as study sites as they have at least one groundwater source with levels of arsenic in excess of 50 µg L^{-1} (Ahmad, 2004) (Figure-4.1). These sites consisted of 1776 households, with a population of 15647 (51% men; 49% women) and an average of 7 family members per house (Pakistan Bureau of Statistics, 1998). A sample size of 398 individuals from 223 households was recruited to the project, derived from a formula for estimating sample proportions from large populations (Collett, 2003). A 95% confidence level and standard error of 0.05, as recommended by Collett (2003), assumes a statistically significant sample size of 384 respondents for a large population.

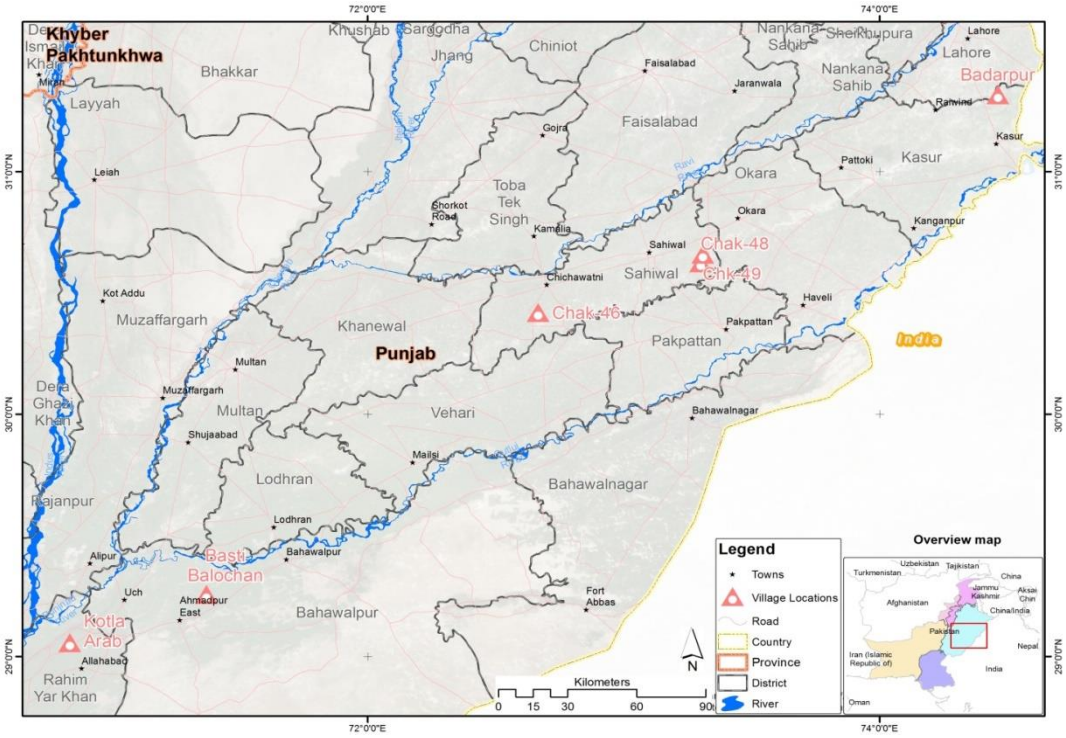

Figure 4.1: Location map of the study area and sampling points
Villages Chak-46/12-L, Chak-48/12-I and Chak 49/12-I in district Sahiwal; Village Badarpur in district Kasur; villages Basti Kotla Arab and Basti Balochan RYK and Bahawalpur districts

The study was conducted in accordance with national and international guidelines for the protection of human subjects and the research protocol was approved by the University of Leeds Research Ethics Committee and National Bioethics Committee of Pakistan (Appendices 3.1 and 3.2). Study participants were recruited (Appendix 3.5) during June-September 2014 by a field team fluent in English and the relevant local languages. Each participant completed a questionnaire with three sections: demographic features (age, sex, body weight, occupation, number of family members), 24-hour food intake diary and 24-hour water intake diary, and each household was supplied with appropriate kitchen utensils (glass: 200-250 ml, cups: 100-200 ml, plates: 150-400 g, and bowl: 100-300 g) with capacity measured and recorded by the field teams. The intake diaries used a semi-quantitative Food Frequency Questionnaire (FFQ) based on the 24 hour recall method (European Food Safety Agency and Allergies, 2010) (Appendix 4.1).

Water intake was calculated based on direct water sources (plain drinking water only) and indirect (water consumed in tea, lassi, and staple food such as rice, wheat and pulses) (Calderon et al., 1999; Ohno et al., 2007; Watanabe et al., 2004). Estimates of water volume provided by the U.S. Department of Agriculture's (USDA) National Nutrient Database were used to calculate indirect water intake (Agricultural Research Service, 2014) and were then combined with direct water intake estimates to make the total water intake. Equations 1-10 (Appendix 4.2) show how the diary information was used to determine daily intakes across the sample population.

4.2.2 Risk assessment methodology

Water and food intake rates were used to calculate carcinogenic risk of arsenic exposure using the United States Environmental Protection Agency (USEPA) human health risk assessment model (Equations 4.1 and 4.2). Risk calculations pertain to the villages and settings from which the primary water and food intake data were obtained. Mutagenic chemicals sometimes cause cancer by a mutagenic mode of action (MOA) which theoretically can lead to a 10 fold greater potency in the first 2 years of life and a 3 fold greater potency between ages 3 and 16 years of age (US Environmental Protection Agency, 2005). This may pose

a higher risk of cancer when exposure occurs during early life. In such cases, age-dependent adjustment factors (ADAFs) are used to assess the additional risk. Applying ADAFs, three main age groups (i.e. 3–6 years, 6–16 years, and >16 years) were used to quantify less than life time and life time cumulative cancer risks (US Environmental Protection Agency, 2011b).

$$\text{Average daily dos (LADD)} = \frac{\text{C x IR} \times \text{ED x EF}}{\text{BW} \times \text{ATe}} \qquad \textit{(Eq.4.1)}$$

$$\text{Cancer risk(CR)} = \text{LADD} \times \text{CSF} \times \text{ADAF} \qquad \textit{(Eq.4.2)}$$

Whereas;

C	Arsenic concentration: water (µg L^{-1}), rice/wheat (µg g^{-1}) (for unit consistency multiplied by 0.001 to get water as (mg L^{-1}) and rice/wheat as (mg kg^{-1})
IR	Ingestion rate: water (L day^{-1}), food (g day^{-1}) (for units consistency multiplied by 0.001 to get food as (kg day^{-1})
EF	Exposure frequency (days $year^{-1}$)
ED	Exposure duration: during life stage (years)
ATe	Average life expectancy (days) = 365 days/year x 67 years
BW	Body weight during life stage (kg)
CSF	1.5 per mg kg^{-1} body weight per day—the cancer slope factor (CSF) for oral ingestion of arsenic (Agency for Toxic Substances and Disease Registry, 2007)
ADAF	Age dependent adjustment factor

Two approaches were used to determine cancer risks: point estimates of cancer risks using intake values from USEPA, World Health Organization (WHO) and regionally appropriate intake values to assess the importance of dietary consumption patterns specific to the population in question (Table 4.2), and a probabilistic approach using the intake values from this study population. For this later risk assessment approach, a Monte Carlo simulation of 10,000 iterations was carried out. In this case, the input parameters defined as probability distributions are given in Table 4.2, and output is likewise presented as a probability distribution (US Environmental Protection Agency, 2001a).

Table-4.2: The input parameters used in calculation of arsenic attributable cancer

Input variable	Unit	Study area	Fitted distribution		values	Data source
			Point estimates	Probabilistic estimates		
As_{water}	µg L^{-1}	17 districts	mean	--	>10, >50 and >100 for point estimate	The World Health Organization (1993), Pakistan Standards Quality Control Authority (2010), Tahir and Rasheed (2014), Ahmad (2004)
			--	**Generalized Pareto (GP) Distribution k = 0.288 σ = 30.112 Θ = 10	>10 for probabilistic estimates	
$As_{raw\ rice}$	mg kg^{-1}	10 districts	mean	mean	0.082 ± 0.054	Rasheed et al. (2016)
As_{Wheat}	mg kg^{-1}	12 districts	mean	mean	0.012	Al-Othman et al. (2016)
Water intake (WI)	L day^{-1}	Study area	mean	mean values fitted with respect to age groups	*Children Age 3-6 years: 1.9 Age 6-16 Years:2.9 Adults >16 Male:3.9 Female:3.2 Overall mean 3.6	Present study
		Other	95th Percentile	NA	*Age 3-6 years: 0.33 *Age 6-16 Years: 0.5 Adults>:2.5	US Environmental Protection Agency (2011a)
			mean	NA	*Age 3-6 years: 1 *Age 6-16 Years:1 Adults >16: 2	World Health Organization (2011)
Rice intake rate (RI)	g day^{-1}	Pakistan	mean	mean	*Children Age 3-6 years: 91 Age 6-16 Years:272 Adults >16	Present study

Input variable	Unit	Study area	Fitted distribution		values	Data source
			Point estimates	Probabilistic estimates		
					Male: 576 Female: 463 Overall mean: 469	
		Bangladesh	constant	NA	Male mean: 1789, Female mean: 1522 Children mean: 862	Khan et al. (2009)
		India		NA	Children: 400 Adults: 750	Roychowdhury et al. (2002)
		USA	constant	NA	Mean:172.6	U.S. Food and Drug Administration (2016)
		Europe	constant	NA	Mean: 175	European Food Safety (2014)
Wheat intake (WhI)	g day^{-1}	Pakistan	mean	mean	Children Age 3-6 years: 149 Age 6-16 Years: 227. Adults >16 Male 426 Female 358 Overall mean 402	Present study
		Bangladesh	mean	NA	Male: 179 Female: 131	Watanabe et al. (2004)
		China	mean	NA	Children:13 Adults:44	Zeng et al. (2015)
		Europe	mean	NA	Mean: 182	Food and Agriculture Organization (2013)
		USA	mean	NA	Mean: 48 (Recommended)	U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015)
Body weight (BW)	kg	Study area	mean	NA	*Children	Present study

Input variable	Unit	Study area	Fitted distribution		values	Data source
			Point estimates	Probabilistic estimates		
					Age 3-6 years: 12 Age 6-16 years: 26 Adults >16 Male: 68 Female: 55 Overall mean 63	
			NA	Fourier Fit of Log (body weight) with respect to log (age)	Refer to Appendix 4.8	
Exposure duration (ED)	years	Study area	constant	Age 3-6 years: 6-Age (picked by Monte Carlo) Age 6-16 Years: 16-Age (picked by Monte Carlo) Adults >16 Year: 67- Age (picked by Monte Carlo)	*Children Age 3-6 years: 3 years Age 6-16 Years: 10 years Adults >16 Age 16-67 years: 51 years Overall ED: 64 years	Present study
Average Life expectancy	years	For all areas	constant	constant	67 (WHO data for Pakistan)	World Health Organization (2015)
Age	years	Study area	mean	--	*Children *Age 3-6 years *Age 6-16 Years Adults Age: 16 to >67 years	Present study
			--	Rician distribution	s (noncentrality parameter) = 27.4061 sigma (scale parameter) = 20.1825	
Averaging Time (AT)	days/ years	For all participants	constant	constant	365	United States Environmental Protection Agency (2011)

Input variable	Unit	Study area	Fitted distribution		values	Data source
			Point estimates	Probabilistic estimates		
Age dependent adjustment factor (ADAF)			constant	constant	For 0-2 years = 10 For age 2-16 years =3 For age 16-67 years = 1	United States Environmental Protection Agency (2011)
Reference dose (RfD)	mg kg^{-1} day^{-1}	For all participants	constant	constant	0.0003	United States Environmental Protection Agency (2011)
Cancer slope factor (CSF)	$(mg/kg\text{-}day)^{-1}$	For all participants	constant	constant	1.5	Agency for Toxic Substances and Disease Registry (2007)

*Results of children are presented in two age groups due to difference in mean body weights, **k: shape parameter,σ: scale parameter, and θ: threshold parameter,

To calculate lifetime risk (cumulative risk) for a population with an average life expectancy of 67 years, the risk calculated for each of the age groups was summed after applying recommended ADAFs. Thus, the life time cancer risk is calculated for a total period of 64 years, starting at the minimum age of the study participants (3 years old). This will also help us determine lifetime risks based on exposure beginning very early compared with those that begin later in life for this region.

Cancer risks for water and most frequently consumed food stuffs i.e. wheat and rice were used to estimate cumulative as well as relative cancer risk from water and food. The USEPA acceptable cancer risk (CR) range is 10^{-4} to 10^{-6} which is dependent on the size of the target population (US Environmental Protection Agency, 2001b). As population size of six villages comprised of 15647 villagers, thus the USEPA's preferred risk goal (1.0×10^{-4}) was considered to rule out even the low risk.

4.2.3 Statistical analysis

The results of the household surveys and cancer risks were analysed using Microsoft Excel and SPSS 17.0 (IBM, New York, NY, USA) for descriptive statistics, two way analysis of variance (ANOVA), Pearson partial correlation analysis and independent samples t-test to identify inter-relationships within the parameters.

4.3 Results and Discussion

4.3.1 Estimation of total water intake

The 398 study participants included 249 men and 149 women; 66 participants <16 years of age (children) and 332 participants ≥16 years (adults); 67 persons < 35 kg body weight (mean body weight at 16 years of age) and 331 were ≥ 35 kg (Appendix 4.3).

The average daily total water intake (direct plus indirect) across this sample population was determined to be 3.5 ± 1.0 L day^{-1} for all participants irrespective of age and sex (Table 4.3). Adult men (3.9 ± 1.0 L day^{-1}) and adult women (3.2 ± 0.7 L day^{-1}) of age ≥16 years consumed more water than children <16 years (2.8

± 0.7 L day^{-1}). The overall average daily total water intake (3.5 L day^{-1}) comprised of 2.7 L day^{-1} (76% of total) of direct drinking water and 0.8 L day^{-1} (24%) of indirect water intake from food and other beverage sources: this was broadly consistent for males and females although children consumed less total, direct and indirect water than adult men and women. From an indirect water intake perspective, lassi and other dairy drinks contributed the most at around 42% followed by rice (21%), tea (18%), pulses (11%) and wheat chapatti (8%) (Appendices 4.4 and 4.5).

Table-4.3: Summary of average daily total, direct and indirect water intake of the study population 95% Confidence Interval

Sex	Age groups (Years)	n	Direct Water Intake (L person^{-1} day^{-1})				In-direct Water Intake (L person^{-1} day^{-1})				Total Water Intake (L person^{-1} day^{-1})			
			Mean	SD	LB	UB	Mean	SD	LB	UB	Mean	SD	LB	UB
Children	3-6	5	1.6	0.498	0.992	2.228	0.3	0.469	0.255	0.909	1.9	0.943	0.766	3.107
	6-16	61	2.3	0.494	2.219	2.472	0.6	0.391	0.476	0.677	2.9	0.660	2.752	3.090
	Overall < 16	66	2.3	0.528	2.160	2.419	0.6	0.399	0.459	0.656	2.8	0.725	2.669	3.025
Male	≥16	206	2.9	0.862	2.794	3.029	1.0	0.464	0.888	1.015	3.9	0.988	3.728	3.998
Female	≥16	126	2.4	0.541	2.307	2.496	0.8	0.371	0.709	0.838	3.2	0.692	3.054	3.296
Average intake (irrespective of sex)	≥16	332	2.7	0.795	2.632	2.804	0.9	0.439	0.837	0.931	3.6	0.947	3.500	3.704
Average intake	All participants	398	2.6	0.773	2.571	2.723	0.8	0.449	0.786	0.874	3.5	0.956	3.383	3.571

SD: Standard deviation, n: No. of samples, LB: lower bound, UB: upper bound

Table-4.4 Average daily food intake (g day^{-1} person^{-1}) of children and adults at 95% Confidence Interval

Sex	Age Group (Years)	Wheat intake			Rice intake			Pulses intake			Vegetable intake			Chicken intake			Total Food Intake		
		Mean ± SD	LB	UB	Mean ± SD	LB	UB	Mean ± SD	LB	UB	Mean ± SD	LB	UB	Mean ± SD	LB	UB	Mean ± SD	LB	UB
Children	3-6	149 ± 81	69	229	91± 7	85	98	75 ± 0	75	75	50 ± 0	50	50	150 ± 71	52	248	292 ± 102	202	382
	6-16	227± 58	212	242	272 ± 97	240	305	154 ± 58	133	176	104 ± 33	93	116	175 ± 45	149	201	526 ± 178	481	571
	Overall <16	222 ± 62	207	237	253 ± 107	219	287	149 ± 59	127	170	103 ± 34	91	115	171± 47	147	196	508 ± 184	464	553
Male	> 16	426 ± 100	412	439	576 ± 175	538	614	252 ± 67	238	266	187 ± 59	175	200	169 ± 47	157	181	888 ± 269	852	925
Female	> 16	358 ± 101	341	376	463 ± 161	418	507	250 ± 73	232	268	181 ± 65	163	199	157± 50	138	176	773 ± 232	732	813
Average intake (irrespective of sex)	> 16	402 ± 105	389	412	532 ± 177	502	563	251± 70	240	262	185 ± 61	175	195	165 ± 48	155	175	844 ± 261	816	873
Average intake	All	372 ± 119	360	384	469 ± 202	439	500	234 ± 78	223	246	170 ± 65	160	180	166 ± 48	157	175	789 ± 279	761	816

The mean total water intake of this study, 3.5 L day^{-1}, was found to be higher than most of the regional studies conducted in Canada, USA, Europe, Latin American and Asian Countries (Appendix 4.6) except those reported by Hossain et al. (2013), Pokkamthanam et al. (2011) and Milton et al. (2006). Water intake differences might be due to regionally specific features as well as the use of different methodologies/definitions of intake values (such as using two different studies to calculate direct and indirect intake separately (Hossain et al., 2013). Within South Asia, all of the studies undertaken in Bangladesh have quantified daily total water intake based on drinking water only (Appendix 4.6) whereas, in India, Pokkamthanam et al. (2011) calculated an average total water intake of 4.5 L day^{-1} (4.8 ± 2.5 L day^{-1} for males and 3.3 ± 1.6 L day^{-1} for females) based on direct and indirect water intake (beverages and food).

Data that do exist in similar geographical regions, for example South Asia, showed considerable variation in water intake both within and between populations. A difference of 1 L day^{-1} between total water intake of the present study and that of Pokkamthanam et al. (2011) might be explained by differences in ambient temperature, dietary habits and/or different cultural practices that exist in India and Pakistan. These reasons may also explain the differences seen in comparison to dissimilar geographic regions: direct only intake values of 1.06 L day^{-1} Kant et al. (2009) and 1.1 L day^{-1} (Barraj et al., 2009) determined for the US population are lower than the present study (2.7 L day^{-1}) possibly due to different climatic and socio-economic conditions (including job types and working patterns), and different food and beverage (e.g. carbonated drinks) intake patterns and preferences.

Drewnowski et al. (2013) reported an US average total water intake of 3.5 L day^{-1} (age group 20 to ≥71 years), of which 37% was from direct drinking water and the remainder (63%) deriving from indirect water intake as hot or cold beverages. This is almost the reverse of the situation reported in this study which puts indirect water intake at 24% of total consumption, similar to the 36% reported by Hossain et al. (2013) in India and the USA study by Ershow and Cantor (1989) which reported 43% from indirect sources and 57% for direct water. This latter study found broadly the same level of indirect water consumption as the present study: 0.88 L day^{-1} (Ershow and Cantor, 1989) compared to 0.8 L day^{-1} although levels

of direct water intake were lower as would be expected due to different climatic, social etc. factors. The role of climate, in particular temperature, in total water consumption is borne out by a number of studies in countries with high ambient temperatures reporting the highest intake levels e.g. 4.5 L day^{-1} in Mexico (Del Razo et al., 2002), 13.2 L day^{-1} in India (Pokkamthanam et al., 2011), and 6-10 L day^{-1} in Bangladesh (Watanabe et al., 2004, Khan et al., 2009, Chowdhury et al., 2000) as well as this study via the village with the highest ambient temperatures, Chak-48/12-I,which had a maximum total water intake of 4.5 L day^{-1} (for a children) and 7.4 L day^{-1} (for an adult).

4.3.2 Estimation of food intake pattern

An analysis of dietary choices and consumption frequency of key staples (wheat, rice, pulses, vegetables and chicken) by the study population over the 24 hour study period found that wheat chapattis were the most popular staple, consumed by 99% of participants, followed by pulses and rice at 42-47%; vegetables at 41% and chicken at 26% (Table 4.4). Consumption of cooked rice was found to be higher in this study, at 469 g day^{-1}, than levels reported in USA, Europe, Africa, Middle East, and Latin America, where rice is not generally considered a staple food, but is broadly consistent with intake levels in South Asia with levels of 400-1789 g day^{-1} reported for Bangladesh and 450-1391 g day^{-1} in India (Signes et al., 2008; Meharg and Rahman, 2003) (Appendix 4.7).

Average daily wheat intake by adults determined from this study (402 g day^{-1}) was found to be higher than in studies reported for USA, Europe and Asian sub-regions (Appendix 4.7). However, wheat has been reported to be the staple food in Pakistan (Prikhodko and Zrilyi, 2013). Previous risk assessment studies have not identified rice, wheat, vegetables, animal products and pulses intake values for Pakistan, either because these have not been considered in the study or the methodology has precluded inclusion. Thus, risk assessment studies have relied mostly on dietary consumption data from other geographical regions. For instance, Rehman et al. (2016) have conducted an arsenic risk assessment using the vegetable intake values reported for Jiangsu Province, China by Jiang et al. (2015).

4.3.3 Factors influencing dietary variations

As has already been noted, there is a difference in water consumption between men and women and between different age ranges. A two-way ANOVA found significant differences (*$p<0.001$ to ≤ 0.05*) between water and/or food intake and mean body weights (male: 68 kg and female: 56 kg), sex, age and villages. The most significant relationships were for sex and age, and can be linked to employment patterns identified by the sociodemographic questionnaire, supporting the association between labour and dietary intake already identified (World Health Organization, 2007). Water consumption increased for men with age up to around 60 years (from 2.22 L day^{-1} to 2.75 L day^{-1}) and then fell (to around 2.52 L day^{-1}) possibly associated with physical labour in the crop fields: 47% of male participants were involved in agricultural activities and these individuals reported the highest levels of water consumption (3.86 L day^{-1}) as shown in Table 4.5. Women identifying as housewives (25% of the surveyed population) had a mean total water intake of 3.28 L day^{-1}.

Table-4.5 Average daily total water intake of various occupational categories

Category	Occupation	Count	Mean total water intake (L day^{-1} $person^{-1}$)
Labour intensive	Masonry workers	2	5.35
	Driver	1	3.91
	Farmers and agriculture labours	186	3.86
	Tailor	4	3.69
	Security Guard	1	3.55
Non-Labour intensive	House Wife	101	3.28
	Student	75	2.93
	Health Worker	1	2.69
	Police Man	1	1.90
	Homeopath Doctor	1	3.40
	Teacher	4	2.90
	Others (including old aged participants and non-school going children)	18	3.25
	NA including infants	3	1.50

4.3.4 Role of water intake values for cancer risk assessment

Human health risk assessment studies (Shah et al., 2012; Muhammad et al., 2011; Muhammad et al., 2010; Khan et al., 2015) undertaken in Pakistan have used USEPA's (1997) default water intake (2 L day^{-1}) and body weight (72 kg) values. This study has demonstrated that water intake was generally higher in the rural population of Pakistan than the revised United States Environmental

Protection Agency (2011) default water intake (2.5 L day^{-1}: updated from 2 L day^{-1} in 2011) with an average daily total water consumption of 3.5 L day^{-1} (men: 3.9 L day^{-1}, women: 3.2 L day^{-1}, children: 2.8 L day^{-1}). This difference in per capita drinking water consumption might contribute to considerably higher risks resulting from exposure to chemical contaminants in water. Using arsenic as an example, higher water intake levels might increase risk estimates for rural populations affected by arsenic-contaminated groundwater. To assess the impact of using default or generic as opposed to population specific intake levels, cancer risk assessment (Table-4.1: Equation-4.2) was carried out using intake variables (Table 4.2) from the present study and compared to USEPA default (2011) and WHO recommended (2011) values. The only difference between the three scenarios (called present study; USEPA and WHO) is water intake (Table 4.2). The results of the risk assessment are provided in Table-4.6. Three risk levels were defined on the basis of risks above maximum allowable concentrations of 10 µg L^{-1} (WHO, USEPA), 50 µg L^{-1} (Pakistan Standards Quality Control Authority, 2010) and reported levels of >100 µg L^{-1} for arsenic concentration in drinking water (Table 4.2).

Table-4.6: Lifetime (Cumulative) Cancer risk point estimates of arsenic intake from water using input variables from the present study, USEPA and WHO

Water Intake data source	Parameters	Statistics	Children		Overall Adults (16-67 years)
			3-6 years	6-16 years	
Pakistan (Present study)	Study participants	n	5	61	332
	ADAF		3	3	1
	Body weight (kg)	mean	12	26	63
		SD	3	8	15
	Age-wise exposure duration	years	3*	10	51
	CR level-1	mean (LB, UB)	0.0017 (0.0014, 0.0017)	0.0014 (0.0011, 0.0014)	0.0006 (0.0006, 0.0006)
	CR level-2	mean (LB UB)	0.0087 (0.0072, 0.0088)	0.0070 (0.0057, 0.0072)	0.0033 (0.0032, 0.0034)
	CR level-3	mean (LB, UB)	0.0173 (0.0142, 0.0176)	0.0141 (0.0110, 0.0143)	0.0065 (0.0063, 0.0067)
USEPA**	CR level-1	mean	0.0006	0.0006	0.0005
	CR level-2	mean	0.0032	0.0029	0.0023
	CR level-3	mean	0.0064	0.0058	0.0045
WHO**	CR level-1	mean	0.0008	0.0006	0.0004
	CR level-2	mean	0.0039	0.0031	0.0018
	CR level-3	mean	0.0079	0.0062	0.0036

*minimum age of study participants
CR: Cancer risk
CR level-1 (>10 µg L^{-1}); CR level-2 (>50 µg L^{-1}); CR level-3 (>100 µg L^{-1})
** SDs not available for USEPA default and WHO recommended water intake values.

Cumulative cancer risks for an exposure duration of 3 to 67 years at all three risk levels and using three different water intake data sources (present study, USEPA and WHO) were found to be above the acceptable USEPA cancer risk criteria of 1.0×10^{-4} (i.e. 1 case of cancer per every 10,000) (Table 4.6). The, lifetime (cumulative) cancer risk at all three risk levels was found to be highest when applying total water intake values from this study (i.e. at lowest risk level, early life exposure with 17 chances in a population of 10000 children of age 3-6 years, 14 children in 10000 of age 6-16 years and 6 men or women in a population of 10000).

Whereas, cancer risk with USEPA default water intake (at lowest risk level, 6 chances in a population of 10000 children of both age groups 3-6 and 6-16 years, later age risk of 5 men or women in 10000 having 51 years of exposure (starting from 16 and continued to 67 years) and with WHO recommended water intake demonstrated an early age exposure of 8 in 10,000 children of 3-6 years, 6 in 10,000 children of 6-16 years and 4 in 10,000 adults, were found to be lower than this study (Table 4.6). Similarly cancer risk at risk levels 2 (>50 µg L^{-1}) and 3 (>100 µg L^{-1}) applying water intake from the present study compared to USEPA default and WHO recommended water intake values (Table 4.2) were revealed to be the highest for all age groups suggesting the significance of population specific water intake for cancer risk estimation.

These findings suggest that using the USEPA default water intake (i.e. 2.5 L day^{-1} for adults or 0.3-0.5 L day^{-1} for children aged 3-16 years) in regions having higher water intake than USA/Europe (e.g. South Asia, Africa etc.) may underestimate cancer risks and, conversely, for lower intake areas, the results might be over-estimated. USEPA default water intake values are based on the National Health and Nutrition Examination Surveys (1999–2010) but are used for worldwide risk assessment studies despite being lower than water intake values for warmer and developing areas of the world. Even in certain warmer parts of USA (i.e. California, Arizona) or during summer seasons, people may drink 4 to 4.5 L day^{-1} (US Environmental Protection Agency, 2000, US Environmental Protection Agency, 1997). Thus, the USEPA default value (2.5 L day^{-1}) or WHO recommendation of (1 L day^{-1} for children and 2.0 L day^{-1} for adults) may underestimate the risks for large numbers of people working in hot and humid

environments (World Health Organization, 2004). Cancer risk was calculated on the basis of total water intake (sum of direct and indirect water intake). Cancer risk determined from present study has also indicated that children are at higher risk than adults suggesting an increased carcinogenic potency during early life stages due to body weight and water intakes differences. This also suggests that lifetime cancer risk for children is much higher due to exposure during early life stages as compared to adults having exposure during later stages in life.

4.3.5 Role of food intake values for cancer risk assessment

In addition to water, food must be considered as an exposure pathway for arsenic although there have been much fewer studies for food than water (Schoof et al., 1999; Tao and Michael Bolger, 1999; Hughes, 2006; Cascio et al., 2011). Human health risk assessments for arsenic in rice require a number of input parameters, such as amount of rice consumed and arsenic concentration in raw or cooked rice.

Past studies have reported rice arsenic levels as 0.32 mg kg^{-1} in France, 0.13-0.16 mg kg^{-1} in Spain, 0.13 mg kg^{-1} in California, 0.2 mg kg^{-1} in Arkansas, USA, 0.33-0.45 mg kg^{-1} in India, and 0.164 mg kg^{-1} in Pakistan (Saleem et al., 1988; Meharg et al., 2007; Bhattacharya et al., 2010). For the purposes of this risk assessment exercise, a conservative arsenic level reported for rice in Pakistan was selected (0.082 mg kg^{-1};Table-4.2; Rasheed et al. (2016)) which is applicable to areas not traditionally associated with high environmental arsenic levels. Therefore, using the average daily rice intake determined in this study compared to intake parameters reported by other studies (Table 4.2) in Equations 4.1 and 4.2 (Table-4.1), it was possible to assess and compare the cumulative cancer risk of consumption of arsenic-contaminated rice (Figure-4.2).

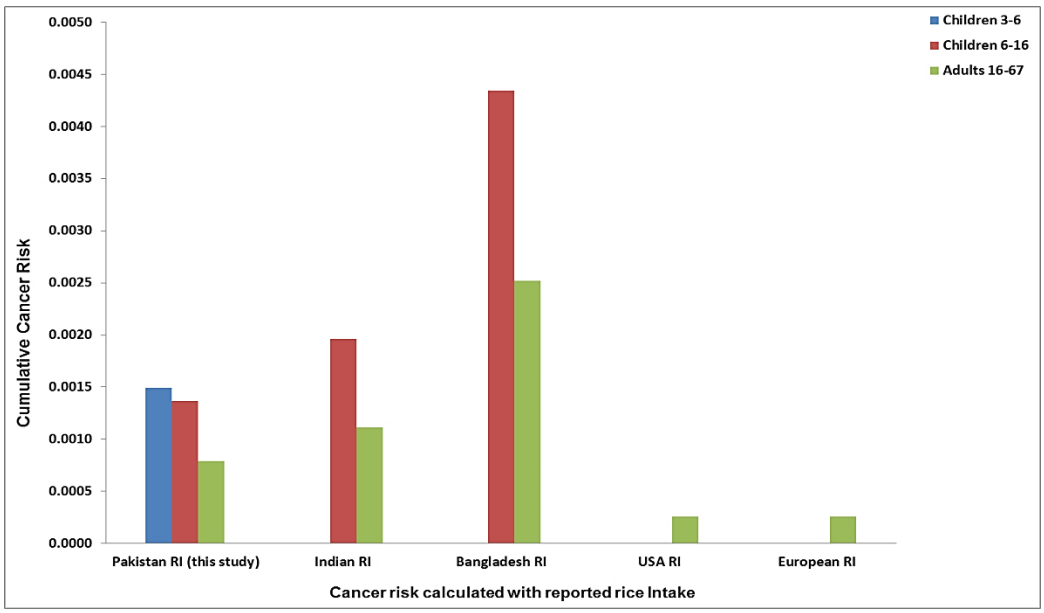

Figure-4.2: Cumulative cancer risk (point estimates at 95% CI) quantified from rice intake values of present study and previously published studies: the only parameter that is changed in each risk assessment is rice intake

Cancer risk due to rice consumption was found to be potentially higher in Bangladesh and India compared to the levels obtained for Pakistan in this study (Figure 4.2) based on differences in rice consumption values. Previous risk assessments for arsenic exposure through rice consumption in India reported risk results closer to this study using Indian intake values i.e. 7 adults in population of 10,000 (Meharg et al., 2009; Mondal and Polya, 2008). Past studies in Bangladesh (Meharg et al., 2009) also report quite similar levels of cancer risk (with 19 women and 22 men in a population of 10,000) in adult life as that shown in Figure 4.2. Cancer risk results using USA/European rice intake (i.e. 3 adults in population of 10,000) were also found to be similar to those identified by Meharg and co-workers (2009). So whilst the mean arsenic concentration used in the calculations is at the lower end of the reported arsenic concentration spectrum, residual cancer risk was still identified: using a higher arsenic concentration level, for instance, use of the recently established advisory limit of 0.2 mg kg^{-1} (or 200 μkg^{-1}) for arsenic in rice would lead to a higher cancer risk. This therefore suggests frequent rice consumption even at low arsenic concentrations may be a significant contributing factor for increased health risks from arsenic exposure. This fact is supported by the work of Banerjee and co-workers (2013), who showed that consuming arsenic-containing cooked rice as a staple food is

associated with elevated genotoxic effects. It is further assumed that the arsenic concentration of raw rice and rice cooking water, volume of cooking water, cooking method and types of rice influence the arsenic level of cooked rice (Ohno et al., 2007). Rinsing, washing and cooking in a high volume of water and discarding excess water were found effective to reduce the inorganic arsenic content of cooked rice by 50% but had no effect on organic arsenic (Raab et al., 2009). In the study area, most of the households had their own ground water source from where water was obtained for drinking, cooking, washing, bathing etc. Higher arsenic levels in their ground water sources is expected as evidenced from previous studies (Tahir and Rasheed, 2014; Mahar, 2015; Shakoor et al., 2015). Thus, rice cooking in a high volume of water was observed to be more prevalent however the arsenic level of cooking water is likely to be a reason for higher dietary arsenic exposure and requires further investigation.

In comparison to water and rice, there are very limited arsenic risk assessment studies for wheat. Studies show that wheat does take up arsenic from soil, indicating that wheat consumption is a potential exposure route (Williams et al., 2007). Arsenic has been identified in wheat grains at levels of 0.02 mg kg^{-1} in USA (Gartrell et al., 1986), 0.05 mg kg^{-1} in Netherlands (Wiersma et al., 1986), 362 mg kg^{-1} in India (Roychowdhury et al., 2002), 0.129 mg kg^{-1} in India (Bhattacharya et al., 2010), 0.127 mg kg^{-1} in Pakistan (Saleem et al., 1988) and 0.175-0.317 mg kg^{-1} in Sindh, Pakistan (Arain et al., 2009). A mean arsenic concentration of 0.012 mg kg^{-1} in wheat grains (Al-Othman et al., 2016) was used in the risk assessments, reflecting a conservative estimate of arsenic concentration for arsenic-affected countries whilst being applicable to regions with lower environmental arsenic levels. Using wheat intake values of this study and those reported for other countries or regions (Table 4.2), cancer risk was found to be within the USEPA acceptable cancer risk range of 1.0×10^{-4} for Bangladesh, China, Europe and the USA intake values. However, for Pakistan, where wheat intake is comparatively higher, cumulative cancer risk was found to be 2 persons (95% CI 0.0002, 0.0002) in a population of 10,000 with exposure initiating during 3-16 years.

4.3.6 Relative cancer risk (point estimates) from water and food sources

Multiple exposures are important when considering overall cancer risk hence it is important to consider the combined contributions made by water (>10 µg L^{-1}) and food to arsenic exposure. Using the water and food intake values (rice and wheat only) of this study, cumulative cancer risk is depicted in Figure 4.3 showing relative risk contribution by total water (51%), rice (44%) and wheat (5%) intake for different sub-populations (Figure 4.3). Food sources like rice are therefore a considerable contributing factor for exposure to waterborne contaminants such as arsenic, so knowledge of intake values (as well as contaminant loading) for different food stuffs is important to elucidate overall cancer risk.

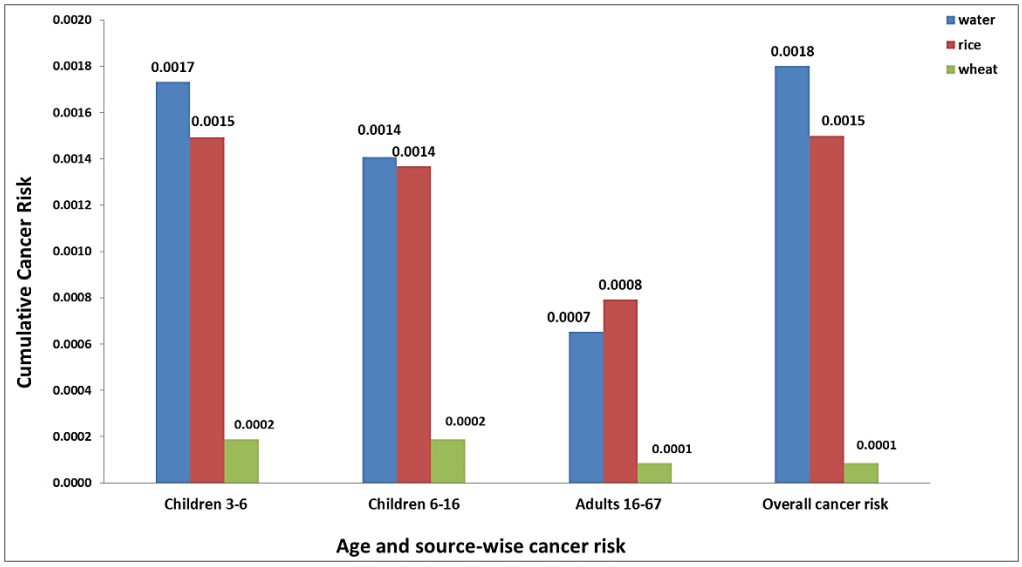

Figure-4.3: Cancer risk (point estimates at 95% CI) based on the average daily water, rice and wheat intake values of present study and exposure duration of 3-67 years of study participants

4.3.7 Probabilistic Risk Assessment approach

4.3.7.1 Results of probability distribution of input parameters

The sample data of arsenic concentration >10 µg L^{-1} of 17 districts (Tahir and Rasheed, 2014, Ahmad, 2004) and age data of 398 study participants were selected to define probability distributions. The optimal fitted distributions of arsenic concentration >10 µg L^{-1} and age of participants were characterised by a

Generalized Pareto distribution and Rician distribution respectively as indicated by a set of parameters (Table 4.7).

Table-4.7 Probability distribution of arsenic in ground water and age of study participants

Probability Distribution	**Arsenic concentration in water**		**Age of study participants**	
	Original Data	**Generalized Pareto distribution**	**Original Data**	**Rician Distribution**
Minimum	10.0	10.0	3	3
Mean	52.5	52.6	36	34
Median	29.4	32.7	36	32
Percentile 95th	166.0	154.4	62	64
Maximum	972.0	809.6	80	83
Standard deviation	63.3	63.7	17	16
Variance	4007.5	4052.7	289	272
Std. mean error	0.926	0.931	0.852	0.826
t-test for equality of means		*p= 0.392*		*p = 0.085*

The body weights of participants were fitted with respect to their ages based on Fourier fit in MATLAB (Appendix 4.8).

4.3.7.2 Probabilistic cancer risk

Probabilistic risk assessment is an improved approach to deterministic cancer risk estimation (point estimation). To better consider the uncertainty inherent in dietary data, probabilistic outputs were associated with seven different age groups as shown in Table 4.8. Using Monte Carlo simulations applied to ADAF transformed data for water, rice and wheat and combined dietary factors (Table 4.8 and 4.9), the results were found to be similar to point estimates with lifetime cancer risk of water and rice higher for intake values determined from this study compared to the USEPA regulatory threshold target cancer risk of 1.0×10^{-4} suggesting probable association between dietary intake and arsenic concentration levels.

Table-4.8 Probabilistic cancer risk (average risk from 10,000 permutations) exposed to arsenic in water at different age groups

Age groups (Years)	Mean	95% CI		SD	Minimum	Maximum	Median	75th percentile	95th percentile
		LB	UB						
3-6	0.0073	0.0061	0.0084	0.0072	0.0016	0.0626	0.0056	0.0093	0.0183
6-16	0.0052	0.0049	0.0056	0.0055	0.0007	0.0624	0.0034	0.0064	0.0152
16-26	0.0042	0.0040	0.0044	0.0047	0.0006	0.0507	0.0027	0.0051	0.0128
26-36	0.0026	0.0025	0.0028	0.0031	0.0004	0.0439	0.0017	0.0031	0.0079
36-46	0.0016	0.0015	0.0017	0.0017	0.0003	0.0283	0.0010	0.0019	0.0045
46-56	0.0010	0.0009	0.0010	0.0011	0.0001	0.0097	0.0006	0.0012	0.0031
56-67	0.0003	0.0003	0.0004	0.0004	0.0000	0.0064	0.0002	0.0004	0.0011

CI: Confidence Interval, LB: Lower bound, UB: Upper bound

Table-4.9 Probabilistic cancer risk (average risk from 10,000 permutations) exposed to arsenic in rice and wheat at different age groups

Age groups (Years)	CR-Rice				CR-Wheat			
	Mean	95% CI		Standard Deviation	Mean	95% CI		Standard Deviation
		LB	UB			LB	UB	
3-6	0.0014	0.0014	0.0014	0.00005	0.0002	0.0002	0.0002	0.00001
6-16	0.0011	0.0011	0.0011	0.00029	0.0001	0.0001	0.0001	0.00003
16-26	0.0010	0.0010	0.0010	0.00020	0.0001	0.0001	0.0001	0.00002
26-36	0.0006	0.0006	0.0006	0.00008	0.0001	0.0001	0.0001	0.00001
36-46	0.0004	0.0004	0.0004	0.00005	0.0000	0.0000	0.0000	0.00001
46-56	0.0002	0.0002	0.0002	0.00004	0.0000	0.0000	0.0000	0.00000
56-67	0.0001	0.0001	0.0001	0.00004	0.0000	0.0000	0.0000	0.00000

CI: Confidence Interval, LB: Lower bound, UB: Upper bound, CR: Cancer Risk

It is interesting to note that highest cumulative exposure from water and food sources initiating at age 3-6 years resulted in the risk probability of 89 children and ranging to 4 adults of age 56-67 in a population of 10,000. The findings are attributed to the incorporation of age dependent adjustment factors (ADAFs) which accounts for adjustment in cancer slope factor according to age. Thus, age adjusted probabilistic cancer risk from food intake of this study population hold a considerable contribution and cannot be neglected in risk quantification process (Figure-4.4 and 4.5).

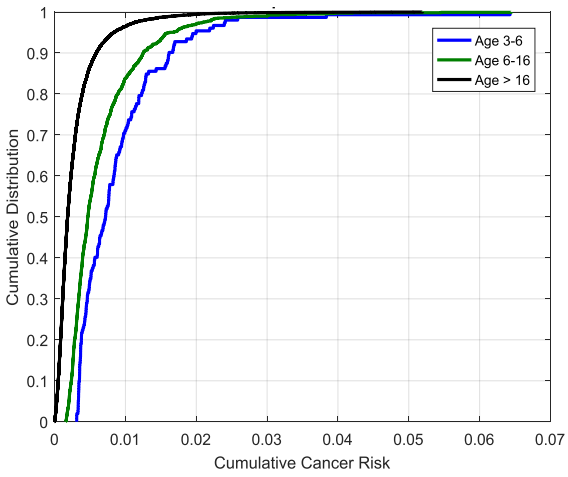

Figure-4.4 Cumulative probability distributions of age adjusted cancer risk from water and food intake for an exposure duration initiating at minimum age of study participant i.e. 3 years proceeding to age 67 years

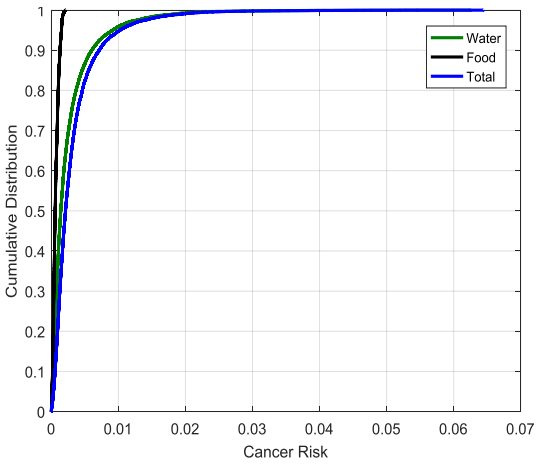

Figure-4.5 Cumulative probability distributions of age adjusted excess lifetime cancer risk from water and food intake (rice and wheat combined) and both (total risk) for the studied population

4.4 Conclusions

Mean total water intake (3.5 L day^{-1}) quantified on the basis of direct plain drinking water (2.7 L day^{-1}) and indirect water from food and beverages (0.8 L day^{-1}) for rural villages in Pakistan was found to be higher than the reported or recommended water intake of many developed countries. Comparison of the intake values determined for Pakistan with the USEPA default and the WHO recommended daily water intake in a cancer risk assessment model revealed a higher total cancer risk of 17 for children of 3-6 years (95% CI 0.0014, 0.0017), 14 for children of 6-16 years (95% CI 0.001, 0.0011) and 6 for adults of 16-67 years (95% CI, 0.0006, 0.0006) in a population of 10,000. This compares to respective figures of 6, 6 and 5 (USEPA) and 8, 6 and 4 (WHO). This difference

at arsenic exposures above 10 µg L^{-1} shows the importance of population specific water intake values and the need to include indirect water sources in risk assessments.

Food is another significant exposure route for chemical risk. Mean average food intake in rural Pakistan was found to be 789 g day^{-1} consisting of wheat (372 g day^{-1}), rice (469 g day^{-1}), pulses (234 g day^{-1}), vegetables (170 g day^{-1}) and chicken (166 g day^{-1}). Consumption of rice was found to be higher than rice intake levels reported in USA (172.6 g day^{-1}), Europe (175 g day^{-1}), but consistent with intake levels reported for Bangladesh (1789 g day^{-1}) and India (862 g day^{-1}). Comparison of the rice intake values determined for Pakistan with these reported rice intake levels in the USEPA cancer risk assessment model revealed a lifetime cancer risk of 15 for children of 3-6 years, 14 for children of 6-16 years and 8 for adults. This compares to figures of 20 for children (6-16 years) and 11 for adults with Indian rice intake or 43 for children (6-16 years) and 25 for adults with Bangladesh rice intake). Using US/European rice intake values the risk for adults is 3) in a population size of 10000. This shows that countries with the highest consumption of rice have potentially higher cancer risks associated with arsenic exposure: India, Pakistan and Bangladesh all have environmental arsenic problems whilst US/European markets might import from these areas. Using wheat intake values from this study (149-402 g day^{-1}) has revealed a total cancer risk of 2 children (3-16 years) and 1 adult of 16-67 years. Whereas, with wheat intake reported for Bangladesh (131-179 g day^{-1}), China (13-44 g day^{-1}), Europe (182 g day^{-1}) and USA (48 g day^{-1}), cancer risk was found to be within the USEPA acceptable cancer risk range of 1.0×10^{-4} highlighting the role of the wheat intake and arsenic concentration level in the risk assessment process (a conservative estimate used). These results are further supported by uncertainty analysis using a probabilistic approach indicating the significance of population specific dietary intake values, arsenic concentrations in water and age of participants in determining cancer risk estimates.

The study findings demonstrate that population specific model values realistically reflect the local situation, whilst also showing that consideration of multiple exposure sources, e.g. water and food sources with respect to age provide a more robust risk assessment. The population specific dietary information from

this study may hold significance for future studies to understand a range of age adjusted dietary exposure risks.

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Chapter 5: Human exposure assessment of different arsenic species in household water sources in a high risk arsenic area

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Abstract

Understanding arsenic speciation in water is important for managing the potential health risks associated with chronic arsenic exposure. Most arsenic monitoring studies to date have only measured total arsenic, with few looking at arsenic species. This study assessed 228 ground water sources in six unstudied villages in Pakistan for total, inorganic and organic arsenic species using ion chromatography inductively coupled plasma collision reaction cell mass spectrometry. The concentration levels approached 3090 μg L^{-1} (95% CI, 130.31, 253.06) for total arsenic with a median of 57.55 μg L^{-1}, 3430 μg L^{-1} (median=52) for arsenate (AsV) and 100 μg L^{-1} (median=0.37) for arsenite (AsIII). Exceedance of the WHO provisional guideline value for arsenic in drinking water (10 μg L^{-1}) occurred in 89% of water sources. Arsenic was present mainly as arsenate (AsV). Average daily intake of total arsenic for 398 residents living in the sampled houses was found up to 236.51 μg kg^{-1} day^{-1}. This exposure estimate has indicated that 63% of rural residents exceeded the World Health Organization's provisional tolerable daily intake (PTDI) of 2.1 μg kg^{-1} day^{-1} body weight. Average daily intake of AsV was found to be 15.63 μg kg^{-1} day^{-1} (95% CI, 5.53, 25.73) for children ≤ 16 and 15.07 μg kg^{-1} day^{-1} (95% CI, 10.33, 18.02) for adults. A mean daily intake of 0.09 μg kg^{-1} day^{-1} was determined for AsIII for children and 0.26 μg kg^{-1} day^{-1} for adults. Organic arsenic species such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and Arsenobetaine (AsB) were found to be below their method detection limits (MDLs).

5.1 Introduction

The natural occurrence of arsenic in ground and surface water poses a health risk for approximately 200 million people globally (Naujokas et al., 2013). Epidemiological studies have indicated an association between chronic exposure to inorganic arsenic via drinking water and cancer of the skin, liver, lung, kidney, prostate and bladder (Agency for Toxic Substances and Disease Registry, 2007b). The toxicity and carcinogenicity of arsenic is strongly associated with its oxidation states and chemical forms. Arsenic species in water consist of inorganic species such as arsenate ($H_2AsO_4^-$ or AsV), arsenite (H_3AsO_3 or AsIII) and organic species like monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB). AsIII was found to be 10 times more toxic than AsV and 70 times more toxic than MMAV and DMAV (Squibb and Fowler, 1983). Higher exposure to inorganic arsenic (iAs) species is reported to be linked with various toxicities including cardiovascular disorders due to oxidative stress (Singh et al., 2011). Organic arsenic species in the trivalent oxidation state (MMAIII and DMAIII) may induce higher cytotoxic and genotoxic effects than pentavalent species (MMAV and DMAV) and inorganic arsenicals due to their higher membrane permeability. This has been exemplified in Chinese hamster ovary cells (Dopp et al., 2004).

Metabolism of inorganic arsenic (iAs) to trivalent methylated arsenic species plays an important role in increasing the toxic effects as MMAIII has shown higher toxicities than AsIII (Petrick et al., 2001; Petrick et al., 2000). Based on these studies, the International Agency for Research on Cancer considers DMA and MMA as possible carcinogens to humans (International Agency for Research on Cancer, 2012b). Despite this, there is no definitive understanding of the mechanism for carcinogenic effects of arsenic species. It is important to measure their concentrations in the environment and biological systems after ingestion to help understand their roles in the development of cancer (Hughes, 2006).

Organic forms of arsenic such as DMA have been used as ingredients in some pesticides such as monosodium methanearsonate (MSMA) or disodium

methanearsonate (Ahuja, 2008; Hughes et al., 2011). Following the identification of organic arsenic species in surface waters or aquifers and associated carcinogenic effects, policy has been developed to limit exposure. For example, the US EPA produced the organic arsenical product cancellation order (US Environmental Protection Agency, 2009) and EU pesticide legislation was developed i.e. Commission Directive 2003/3/EC: Regulation (EC) No 304/2003 (Official Journal of the European Union, 2003). Nevertheless, few studies, particularly in arsenic affected regions, exist on iAs speciation in water (Chen et al., 1994; Bhattacharya et al., 2006). In such regions, exposure assessments of inorganic and organic arsenic species may assist in identifying the likely sources associated with cancer clusters. These may include arsenic contaminated ground water used for drinking, food preparation, cooking and irrigation purpose. Previous studies undertaken in Pakistan have only determined iAs using commercial field testing kits (Mahar, 2015; Uqaili A. A., 2012; Ahmad, 2004) or validated a small percentage of samples in the laboratory for inorganic arsenic (Haque, 2008; Farooqi et al., 2007). Whereas, arsenic speciation studies (Rehman et al., 2016, Brahman et al., 2013, Baig et al., 2016) have only analysed AsIII using simple spectrophotometry or Graphite Furnace Atomic Absorption Spectrometery (GFAAS). AsV has been determined only as the difference between iAs and AsIII, whilst organic arsenic species (DMA, MMA and AsB) have not been analysed in water.

Considering the unknown extent of arsenic species in ground water and uncertainties regarding the species dependent arsenic toxicity, the aim of this study was to conduct an exposure assessment for different arsenic species in the groundwater of six previously unexplored rural settings. The specific objectives were to; 1) assess the spatial distribution of total arsenic (tAs), inorganic (AsIII and AsV) and organic arsenic species (DMA, MMA and AsB) in ground water aquifers; 2) determine the magnitude of arsenic exposure from domestic ground water and associated health implications.

5.2 Methodology

5.2.1 Sampling design and study area characteristics

This study uses a population based probability design within four districts of Pakistan (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan). Six villages within these four districts were selected for sampling, where at least one groundwater source was found to contain arsenic concentrations >50 µg L^{-1}. The prevalence of arsenic associated health symptoms among the native residents of at least 1% of houses was also used. Ground water (obtained from hand pumps and dug wells) is the major water source in the study villages in the alluvial plain of the south-flowing Indus river and its five major tributaries (Pakistan paedia, 2008). These consisted of 1776 households, with a population of 15647 (51% men; 49% women) and an average of 7 family members per house (Pakistan Bureau of Statistics, 1998). The detailed sampling design is published elsewhere (Rasheed et al., 2017). A sample size of 223 households was selected, derived from a formula for estimating sample proportions (Collett, 2003). Accordingly, a 95% confidence level and standard error of 0.05 assumes a statistically significant sample size. Ground water sampling for this study was conducted randomly depending on the willingness of 223 households simultaneously with data collection on daily water intake rate, body weight, and age from 398 residents of such houses. Five additional households were willing to participate only in water sampling; hence a total of 228 water samples were collected.

5.2.2 Samples collection procedure

Groundwater samples were collected from hand-pumps and dug wells at depths of 10 to 31 m following typical practice of purging for 5 to 10 minutes to obtain fresh groundwater. The groundwater samples were collected in duplicate in high density polyethylene (HDPE) bottles (125 mL each). One water sample was filtered and acidified on-site by adding 2 to 3 drops of concentrated nitric acid (HNO_3) to stabilize arsenic and reduce precipitation as recommended by USEPA method 200.8-modified (US Environmental Protection Agency, 1994). The acidified water samples were used to analyse total arsenic (tAs). For

arsenic speciation, the second sample was filtered and preserved with 0.125 M ethylenediaminetetraacetic acid (EDTA) (Garbarino et al. 2002). Samples were kept in an insulated cooler containing ice and transported to the local laboratory for storage at 4 °C. They were then transferred to Brooks Applied Laboratory (BAL), USA by FedEx courier with dry ice under strict quarantine regulations and stored at 4 °C prior to analyses.

5.2.3 Samples processing for total arsenic and speciation

The pH of water samples was measured in the field using a pH meter (Model 350, Jenway), whilst Iron was tested in the laboratory by Phenanthroline method (3500-Fe, APHA, 2012). The tAs concentrations were obtained using an inductively coupled plasma mass spectrometer with dynamic reaction cell (DRC™) technology (USEPA method 200.8, modified). Arsenic speciation data were obtained by analysis of samples using ion chromatography inductively coupled plasma collision reaction cell mass spectrometry (IC-ICP-CRC-MS). Peak integration was performed by automated integration. Chromatographic peaks were integrated using the ICP-MS plasma lab software.

5.2.4 Quality Assurance

The quality of analytical work was checked by the analysis of NIST (National Institute of Standards and Technology) traceable standard reference materials (SRMs-1640A, trace elements in natural water), blanks and duplicates (Tables 5.1). Data quality in terms of precision, accuracy, method detection limits (MDLs), and completeness met the criteria established in the BAL's quality assurance project plan (QAPP), i.e., relative percent difference (RPD) of <25%, percent recovery of 75 to 125% and completeness of 80%.

Table-5.1: Summary of Quality Control Data of six analytical batches

Parameter	Method Detection Limits (MDLs)			Calibration Standard (CAL)		Initial Calibration Verification (ICV)		Duplicate (DUP)		Matrix Spike (MS)		Certified Reference Material (CRMs) NIST 1640a		Laboratory Fortified Blank (BS)		
	% Rec	Results	SD	% Rec	SD	% Rec	SD	% Rec	SD	% Rec	SD	% Rec	SD	% Rec	Results	SD
tAs	84	0.31	0.28	100	3.16	98	8.01	117	2.46	98	10.57	96	6.91	86%	0.62	0.56
AsIII	97	0.36	0.05	104	7.28	108	4.08	106	3.83	103	4.89	-	-	89%	0.78	0.37
AsV	109	0.12	0.03	101	9.5	98	1.63	102	7.66	107	7.72	-	-	98%	1.07	0.16
MMA	90	0.18	0.04	97	7.21	75	14.29	109	4.4	109	7.35	-	-	97%	1.24	0.21
DMA	96	0.27	0.04	103	6.47	113	1.63	106	4.69	106	6.93	-	-	97%	1.1	0.17
AsB	100	0.37	0.03	-	-	107	8.57	-	-	-	-	-	-	99%	1.08	0.09

Expected percent recovery: 75-125%

5.2.5 Arsenic Exposure Assessment

The average daily dose (ADD) of tAs and arsenic species was calculated using Equation (5.1) (US Environmental Protection Agency, 1997).

$$ADD = \frac{C \times IR \times EF \times ED}{AT \times BW} \quad \textit{(Eq. 5.1)}$$

Where ADD is average daily dose (as μg kg^{-1} day^{-1}), C represents the arsenic concentration in ground water (in μg L^{-1}), IR is the drinking water intake rate (L day^{-1}), EF is the exposure frequency (365 days $year^{-1}$); and ED is the exposure duration (years of using the ground water source). BW is the body weight (kg), and AT is the averaging time and is equal to (ED x 365 days/year). For children (≤16 years), the specific age class is considered as the ED.

The chronic daily intake and health risk was assessed for the study population by comparing the individual exposure to the reference level i.e. (RfD) and provisional tolerable daily intake (PTDI) via a ratio known as the "hazard quotient (HQ)". In this study, the HQ is quantified for tAs and iAs species for each study participant recruited in the past study (Rasheed et al., 2017) using Equation (5.2) (US Environmental Protection Agency, 1997).

$$HQ = \frac{ADD}{RfD} \quad \textit{(Eq.5.2)}$$

Where;

HQ	Hazard quotient
ADD	Average daily dose of arsenic from the oral ingestion (μg kg^{-1} day^{-1})
RfD	Reference dose: 0.0003 mg kg^{-1} day^{-1} (US Environmental Protection Agency, 1993) for iAs

ADD values were compared with the World Health Organization's (WHO) provisional tolerable daily intake (PTDI) of 2.1 μg kg^{-1} day^{-1} (World Health Organization, 2010, World Health Organization, 1989). If the calculated HQ is equal to or less than 1, the human health effect is assumed to be negligible, while

a HQ greater than 1 suggests that there may be health concerns (United States Environmental Protection Agency, 2011). To provide a conservative estimate of health risk for this study, the ratio between ADD and the oral RfD set by USEPA and between ADD and PTDI for total arsenic (JECFA/WHO guidelines) were considered. Considering the absence of RfDs for arsenic species, it was assumed that iAs is primarily AsV, hence the RfD of 0.0003 mg kg^{-1} day is also used for AsV. Based on 1.5 orders of magnitude of higher toxicity of AsIII than AsV , an estimated RfD of 0.000006 mg kg^{-1} day^{-1} is used for AsIII (Markley and Herbert, 2009).

5.2.6 Statistical analysis

Arsenic data distributions for total arsenic, AsV and AsIII was found to be positively skewed in this study, hence the data set was normalized by log transformation prior to statistical analysis. Following the log-normal distribution, arithmetic mean (AM), geometric mean (GM), median, upper and lower confidence limits were then calculated. Median and the geometric mean (GM) were expected to better represent the natural level of arsenic in ground water by minimizing. Microsoft Excel and SPSS 17.0 (IBM, New York, NY, USA) were used for generating descriptive statistics and Pearson partial correlation analysis. Nonparametric Pearson's correlation coefficients were used to assess the relationship between concentrations of tAs and arsenic species. Statistical significance was two-tailed and set at $\alpha = 0.05$.

5.3 Results and Discussion

5.3.1 Total arsenic and arsenic species

Statistical observations across the six villages imply non-uniform distribution of tAs, AsV and AsIII in groundwater. This observation is supported by large differences among mean and median followed by positive skewness of the original data (skewness: tAs (4.04), AsV (4.12) and AsIII (4.11). Log-transformation of arsenic concentrations significantly reduced the skewness as 0.34, 0.29 and 1.86 for tAs, AsV and AsIII respectively (Appendices 5.1 to 5.3).

Village-wise summary statistics of tAs and iAs species has shown the median values closer to the central tendency. The highest median concentration for total

arsenic was found to be 1670 µg L^{-1} (95% CI, 1013.91, 2016.67) in groundwater of village Badarpur (n= 16) followed by 154 µg L^{-1} (95% CI, 159.26, 361.16) in village Chak-48 (n=45) and 65.30 µg L^{-1} (95% CI, 53.82, 74.68) in village Chak-46 (n=57) as shown in Table-5.2. Median total arsenic across all samples (n=228) of study area was found to be 57.55 µg L^{-1} (95% CI, 130.31, 253.06) and a range of 0.48 to 3090 µg L^{-1} as given below in Table-5.2**.**

Table-5.2 Summary statistics of tAs and iAs species (µg L^{-1}) in groundwater samples (n = 228)

Analyte	**Statistics**	**Chak-46**	**Chak-48**	**Chak 49**	**Basti Balochan**	**Badarpur**	**Basti Kotla Arab**	**Overall**
No of samples	n	57	45	50	31	16	29	228
tAs	AM	64.25	260.21	57.73	25.16	1515.29	14.52	191.68
	SD	39.30	336.01	26.42	8.35	940.92	13.23	470.31
	GM	49.76	145.29	49.10	23.25	1075.29	9.21	55.33
	Median	65.30	154.00	61.450	25.90	1670.00	11.40	57.55
	95% CI LB	53.82	159.26	50.22	22.19	1013.91	9.49	130.31
	95% CI UB	74.68	361.16	65.24	27.84	2016.67	19.56	253.06
	Minimum	3.56	8.50	7.11	8.25	43.60	0.48	0.48
	Maximum	228.00	1401.05	95.60	37.70	3090.00	51.40	3090.00
AsV	AM	64.52	250.11	46.54	20.72	1690.18	16.48	199.22
	SD	38.99	361.88	29.15	7.32	1051.33	15.57	523.95
	GM	127.18	49.49	34.60	18.88	1198.53	9.59	49.08
	Median	64.00	124.00	46.20	21.20	1855.00	12.60	52.00
	95% CI LB	54.18	141.39	38.25	17.98	1129.97	10.56	130.85
	95% CI UB	74.87	358.83	54.82	22.92	2250.40	22.40	267.60
	Minimum	2.40	7.67	3.01	5.05	47.90	0.11	0.11
	Maximum	222.00	1440.00	106.00	29.60	3430.00	62.50	3430.00
AsIII	AM	0.39	3.79	19.22	1.24	0.91	0.62	5.37
	SD	0.08	11.41	30.05	1.21	0.78	0.51	16.61
	GM	0.38	0.81	3.87	0.83	0.70	0.51	0.88
	Median	0.37	0.37	2.73	0.61	0.60	0.37	0.37
	95% CI LB	0.36	0.36	10.68	0.76	0.49	0.43	3.20
	95% CI UB	0.41	7.22	27.76	1.68	1.32	0.82	7.54
	Minimum	0.37	0.37	0.37	0.37	0.37	0.37	0.37
	Maximum	0.96	57.50	100.00	4.82	3.26	2.27	100.00

n: Number of samples; AM: Arithmetic mean; SD: Arithmetic standard deviation; GM: Geometric mean; 95% CI: Confidence Interval, LB: Lower bound; UB : Upper bound.

The maximum level of tAs in ground water determined in this study is found to be higher than previous arsenic monitoring studies undertaken in Pakistan i.e. 0.2 to

2580 µg L^{-1} (Khattak et al., 2016; Rasool et al., 2015; Mahar, 2015; Shakoor et al., 2015; Rehman et al., 2016; Brahman et al., 2013; Farooqi et al., 2007; Haque, 2008; Nickson et al., 2005). The highest level of iAs discovered in this study is of the same order of magnitude as reported in other studies of arsenic rich zones of the world e.g. Bengal Basin, Argentina, Mexico, northern China, Taiwan and Hungary, where arsenic in ground water was found up to 5000 µg L^{-1} (Smedley and Kinniburgh, 2002). The percentage of tAs exceedance above the WHO provisional guideline value for arsenic in drinking water (10 µg L^{-1}) was found to be highest for the samples collected from the villages of Badarpur and Basti Balochan (100%) followed by Chak-48 (98%), Chak-49 (96%), Chak-46 (91%) and Kotla Arab (54%). 126 sources (56%) were also found to have tAs above Pakistan's water quality standard for arsenic i.e. 50 µg L^{-1} (Pakistan Standards Quality Control Authority, 2010) as depicted in Figures 5.1a to 5.1c.

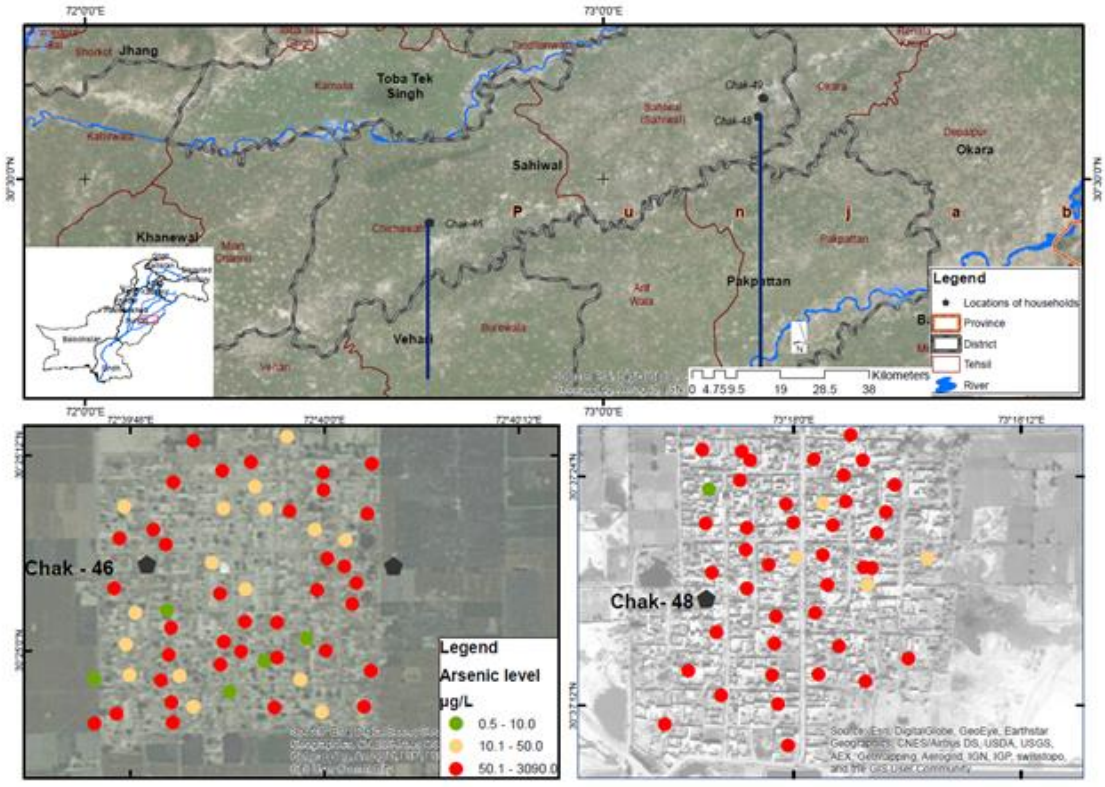

Figure 5.1a: Spatial distribution of tAs in villages Chak-46/12-L (*n=57*), Chak-48/12-I (*n=45*) in district Sahiwal

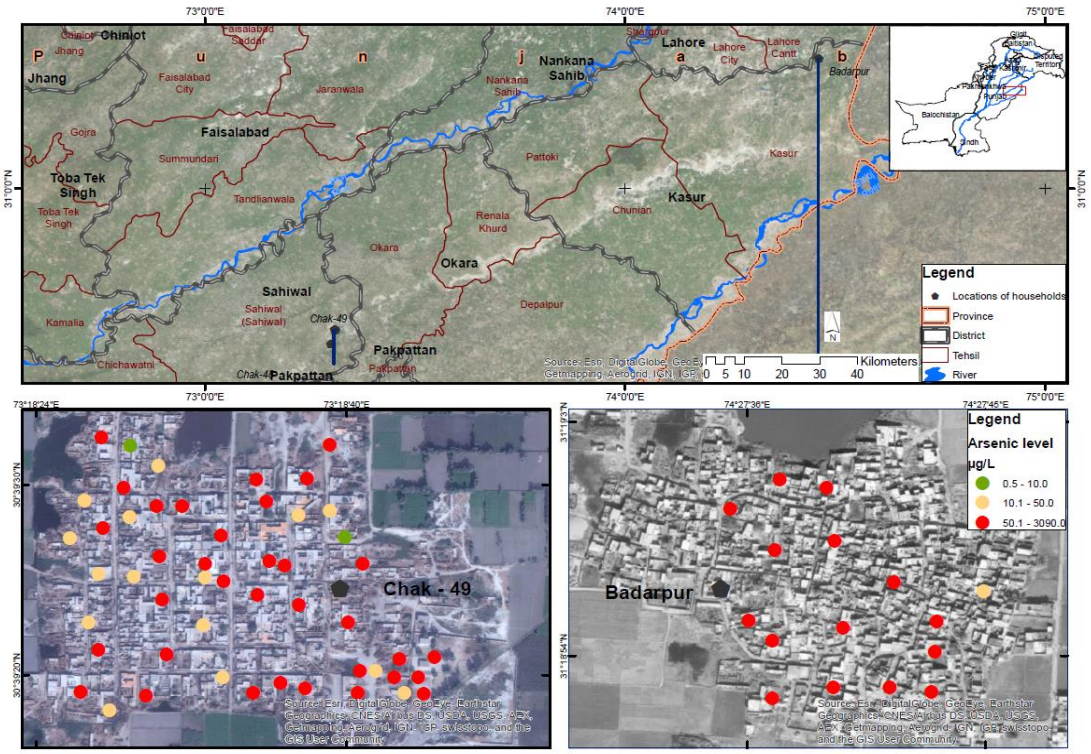

Figure 5.1b: Spatial distribution of tAs in villages Chak 49/12-l (n=50) and Badarpur (n=16) in Sahiwal and Kasur districts

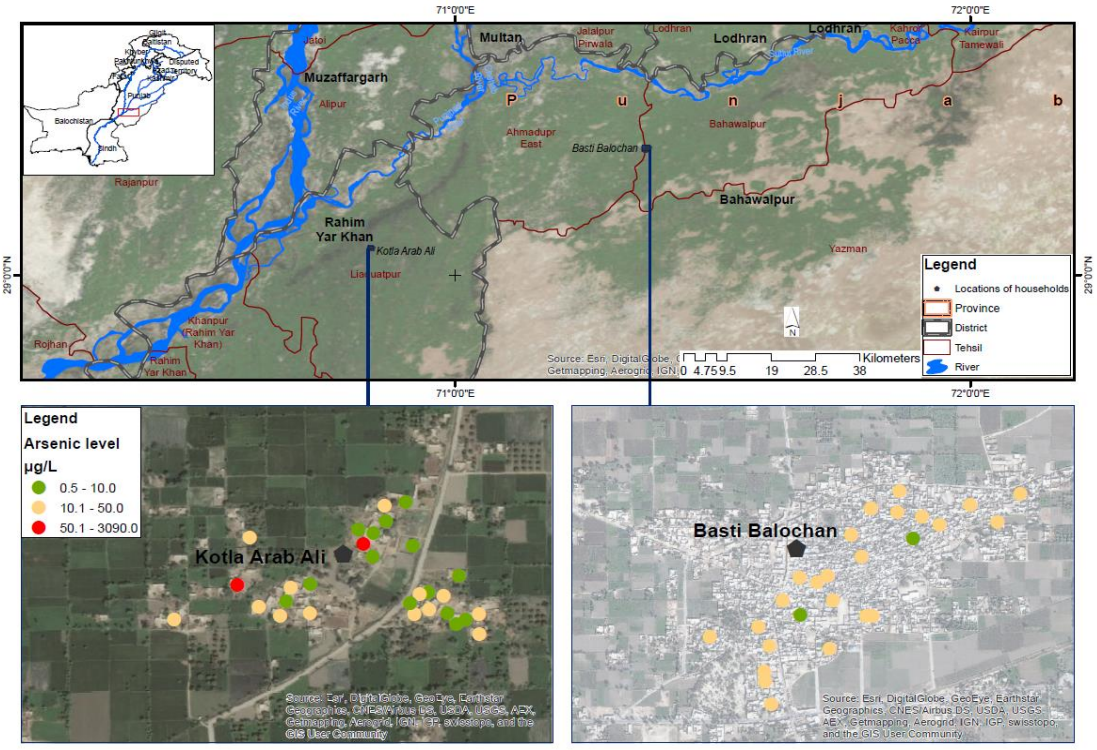

Figure 5.1c: Spatial distribution of tAs in villages Basti Kotla Arab (n=29) and Basti Balochan (n=31) in districts RYK and Bahawalpur

Inorganic arsenic speciation results have shown the median AsV concentration to be 1855.00 µg L^{-1} in Badarpur followed by 124.00 µg L^{-1} and 64.00 µg L^{-1} in Chak-48 and Chak-46 respectively. AsV concentration across all samples ranged between 0.11 and 3430.00 µg L^{-1} with median value being 52.00 µg L^{-1} (95% CI, 130.85, 267.60). AsV was the most dominant iAs species and a strong relationship existed between tAs and AsV (Pearson's r = 0.964, n = 228, 95% CI, 0.929, 0.999).

Following AsV, the second most prevalent inorganic species was AsIII (Table-5.2). Village-wise comparison of AsIII showed a highest median concentration of 2.73 µg L^{-1} in village Chak-49 with an overall range of 0.37 to 100 µg L^{-1} (Table-5.2). The overall median of AsIII was found to be 0.37 µg L^{-1} (95% CI, 3.20, 7.54). There were only 21 water sources discovered with co-existence of AsIII and AsV and out of these, AsIII was dominant in only 13 sources (Figure 5.2).

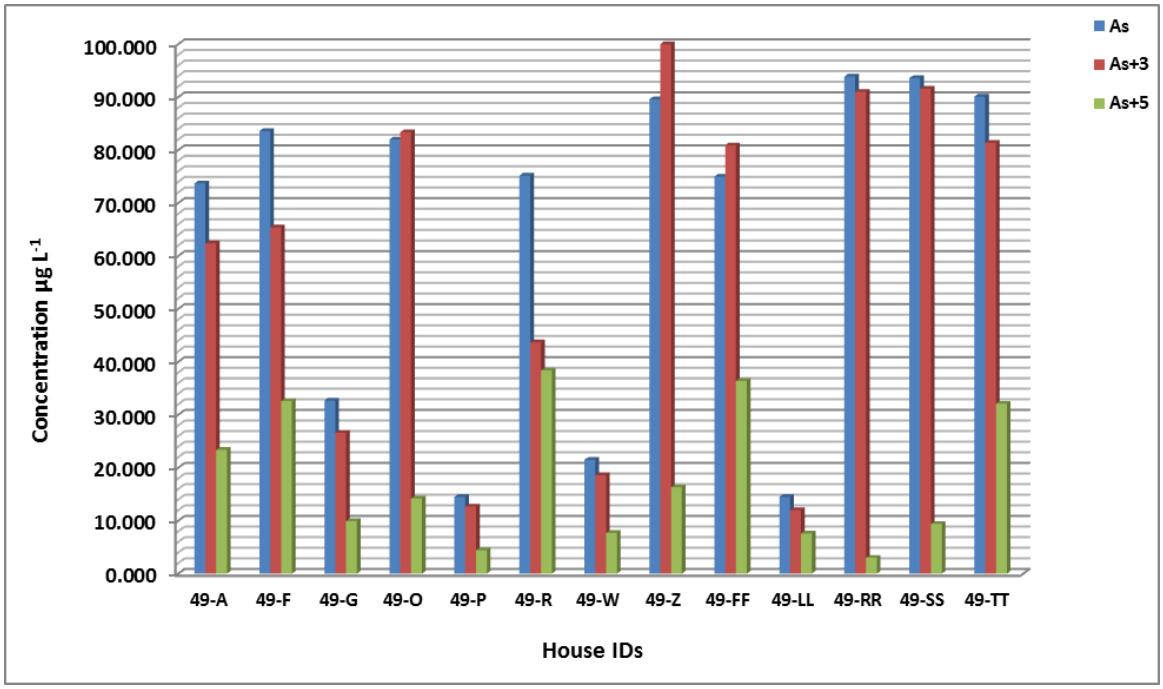

Figure-5.2: Pre-dominance of AsIII (µg L-1) in some groundwater samples (n = 13) indicated by concentration levels of tAs, AsIII and AsV

Other organic arsenic species (MMA, DMA and AsB) were found to be below or close to the method detection limits (MDLs) as shown below in Table-5.3

Table 5.3 Organic arsenic species (µg L^{-1}) in groundwater samples (n = 228)

Villages		AsB		DMAs		MMAs	
	n	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD
Chak-46/12-L	57	0.37-0.37	0.37 ± 0.00	0.28-0.28	0.28 ± 0.00	0.2-0.2	0.20 ± 0.00
Chak-48/12-I	45	0.37-0.37	0.37 ± 0.00	0.28-1.8	0.31 ± 0.23	0.2-0.2	0.20 ± 0.00
Chak 49/12-I	50	0.37-0.37	0.37 ± 0.00	0.28-0.7	0.29 ± 0.06	0.14-0.14	0.14 ± 0.00
Basti Balochan	31	0.37-0.37	0.37 ± 0.00	0.28-0.28	0.28 ± 0.00	0.2-0.2	0.20 ± 0.00
Badarpur	16	0.37-0.37	0.37 ± 0.00	0.28-0.4	0.29 ± 0.03	0.2-0.2	0.20 ± 0.00
Basti Kotla Arab	29	0.37-0.37	0.37 ± 0.00	0.28-0.28	0.28 ± 0.00	0.2-0.2	0.20 ± 0.00
Overall	228	0.37-0.37	0.37 ± 0.02	0.28-1.8	0.29 ± 0.11	0.14-0.2	0.19 ± 0.02

SD: Standard Deviation, Min: minimum, Max: maximum

5.3.2 Geological impact on relationship between arsenic species

The co-existence of AsIII and AsV possibly associated with variations in aquifer's redox conditions was also evidenced by past studies (Bhattacharya et al., 2006; Smedley and Kinniburgh, 2002). However, contrary to the dominance of AsV in this study, AsIII (462 µg L^{-1}) has been found as the principal species in the water sources in Taiwan (Chen et al., 1995; Ko et al., 1997). In West Bengal India, 60% to 90% of total arsenic existed as AsIII (6.8 to 462 µg L^{-1}) and 20% to 60% as AsV (7 to 185 µg L^{-1}) (Shraim et al., 2002). A mixed reduction-oxidation process associated with localized geology was concluded to be responsible for such variations in these past studies. A mean ratio of AsIII to tAs was found to be within the range 0.1 to 1.1. This is slightly higher than typically found in Bangladesh i.e. 0.5 to 0.6 (Department of Public Health Engineering Bangladesh, 1999) and closer to that found (0.7 to 0.9) in reducing groundwater of Inner Mongolia (Smedley et al., 1999).

This study data showed that arsenic in ground water aquifers appeared to increase in concentration from the southern region (district Bahwalpur) towards the central region (district Kasur) of Punjab province. This study area is located within the Indus plain having geogenic presence of quaternary alluvial-deltaic sediments derived from sedimentary rocks (Nickson et al., 2005). Sedimentary rocks due to the slow formation over centuries allows for aggregation of iron with greater capacities for arsenic retention. Under oxidizing conditions (i.e. oxidation-reduction potential >0 millivolts), AsV is generally found to be the dominant form,

whereas, higher concentration of more toxic AsIII in ground water is expected under reducing conditions (i.e. oxidation-reduction potential <0 millivolts) (Sorg et al., 2014). Excessive iron causes the onset of reducing conditions in alluvium (anoxic conditions) resulting in higher mobility of AsIII (Smedley, 2008). Indeed, there was a strong relationship between iron (0.01-1.67 mg L^{-1}) and AsIII in ground water of Chak-49 detected in the current study (Pearson's r=0.954, n=21, 95% CI, 0.755, 1.1533).

Consumption of ground water with an elevated AsIII concentration could make a significant contribution to the intake of toxic iAs species, with possible long-term adverse effects on the human health. However, the WHO provisional guideline value for arsenic in drinking water (10 µg L^{-1}) and any of international or national enforceable regulations do not differentiate among arsenic species.

Arsenic contamination has also been reported to be associated with shallow wells (Mahar, 2015; Brahman et al., 2013; Welch et al., 2000; Ahmad, 2004). This agrees with the current study with presence of arsenic at a depth of 10 to 31 meters. To remediate shallow well contamination, the strategy of development of deeper wells has been the most recommended option for arsenic affected areas. However, the presence of more toxic AsIII has been reported in wells deeper than 170 metres in Taiwan (Tseng et al., 1968, Chen et al., 1994, Guo et al., 1994), Bangladesh (Roychowdhury, 2010) and the Mekong Delta in Vietnam (Erban et al., 2013). Other studies did not find any correlation between arsenic concentration and wells depth (Boyle et al., 1998; Nimick, 1998). The presence of AsIII in 21 shallow wells in this study suggests, nevertheless, that this contamination is not just associated with deeper wells. The transport of arsenic in groundwater is also reported to be influenced by pH (Lovley and Phillips, 1988). However, the pH of ground water in this study was determined to be between 6.50 and 8.10 and there was no significant relationship between iAs species and pH (Pearson's correlation coefficient (r) as = -0.14 (tAs), 0.008 (AsIII) and -0.16 (AsV).

5.3.3 Arsenic exposure assessment

Given the high levels of tAs in drinking water supplies than WHO provisional drinking water guideline value of 10 µg L^{-1}, an exposure assessment was carried

out for the six villages. The principal factors that have been taken into account in the exposure assessment calculations are presented in Table 5.4.

The daily intake of tAs as an average daily dose (ADD) for 398 persons residing within the 223 houses was found to be 15.12 µg kg^{-1} day^{-1} (95% CI, 5.59, 24.66) and 14.18 µg kg^{-1} day^{-1} (95% CI, 10.33, 18.02) for age groups of ≤ 16 and >16 years respectively. Similar mean values were found for AsV whereas, for AsIII, a very low average daily dose is shown in Table-5.4. Compared with the provisional tolerable daily intake (PTDI) value of 2.1 µg day^{-1} kg^{-1} body weight (World Health Organization, 1989) of iAs, 51 of 66 children of age ≤ 16 were found to have an average daily dose (ADD) for tAs above this limit. 201 of 332 adults (>16 years) exceeded the daily intake of 2.1 µg day^{-1} kg^{-1} body weight. As the provisional tolerable daily intake (PTDI) value of 2.1 µg day^{-1} kg^{-1} body weight (World Health Organization, 1989) is set on the basis of iAs, no species based assessments can be made.

Consumption of water with a iAs level below the WHO value (10 µg L^{-1}) has indicated a total daily intake of 0.37 ± 0.26 µg day^{-1} kg^{-1} for tAs which did not exceed the PTDI of 2.1 µg day^{-1} kg^{-1} body weight. However, at a concentration level of 10 to 50 µg L^{-1}, the average daily dose was found to be 2.01 ± 1.32 µg day^{-1} kg^{-1}. While, at an arsenic concentration of 50 to 100 µg L^{-1}, intake was found to be 5.09 ± 2.90 µg day^{-1} kg^{-1} and a higher intake of 59.62 ± 63.32 µg day^{-1} kg^{-1} was found at arsenic concentration levels above 100 µg L^{-1}. These findings have revealed that 63% (n=252) of the household members consuming arsenic contaminated water >10 µg L^{-1} also exceeded the PTDI of 2.1 µg day^{-1} kg^{-1} body weight. These results suggest that countries, including Pakistan, currently following a drinking water standard for arsenic of 50 µg L^{-1} would place many people at risk of developing adverse health effects in rural areas.

Table-5.4: Average daily dose (ADD) of tAs and arsenic species from drinking water at 95% CI

Age groups (years)	n	Statistics	Body weight (Kg)	*Total daily water intake	ED (years)	ADD (µg kg^{-1} day^{-1}) (mean ± SD)						Population > 2.1 µg kg^{-1} bw day^{-1} of total arsenic	
						tAs	AsV	AsIII	MMA	DMA	AsB	n	%age
3-6	5	-	12 ± 3	1.94	5	8.12 ± 5.86	8.37 ± 6.044	0.06 ± 0.02	0.026 ± 0.0056	0.04 ± 0.011	0.06±0.014	5	8
6-16	61	-	26 ± 8	2.92	12	15.70 ± 41.06	16.22 ± 43.485	0.09 ± 0.15	0.023 ± 0.0063	0.03 ± 0.009	0.04±0.012	46	70
≤ 16	66	-	25 ± 8	2.85	12	15.12 ± 39.53	15.63 ± 41.858	0.09 ± 0.14	0.023 ± 0.0063	0.03 ± 0.010	0.04 ± 0.013	51	78
	-	*LB*	-	-	-	5.59	5.53	0.05	0.02	0.03	0.04	-	-
	-	*UB*	-	-	-	24.66	25.73	0.12	0.03	0.04	0.05	-	-
	-	*Min*	9	-	-	0.065	0.01	0.02	0.01	0.02	0.02	-	-
	-	*Max*	44	-	-	195.88	226.59	1.05	0.04	0.06	0.08	-	-
Male >16	206	-	68 ± 14	3.86	20	14.05 ± 33.65	14.73 ± 37.430	0.32 ± 1.01	0.011 ± 0.0045	0.02 ± 0.008	0.02 ± 0.008	144	43
Female >16	126	-	55 ± 13	3.18	20	14.40 ± 39.05	15.64 ± 44.022	0.17 ± 0.67	0.012 ± 0.0035	0.02 ± 0.005	0.02±0.006	57	17
All >16	332	-	63 ± 15	3.6	20	14.18 ± 35.74	15.07 ± 39.997	0.26 ± 0.90	0.011 ± 0.0042	0.02 ± 0.007	0.02 ± 0.007	201	61
	-	*UB*	-	-	-	10.33	10.77	0.17	0.01	0.02	0.01	-	-
	-	*LB*	-	-	-	18.02	19.38	0.36	0.02	0.03	0.02	-	-
	-	*Min*	29	-	-	0.02	0.005	0.01	0.01	0.01	0.01	-	-
	-	*Max*	105	-	-	236.51	262.54	7.57	0.03	0.09	0.06	-	-

bw: body weight, LB: lower bound, UB: upper bound, CI: Confidence interval

The maximum average daily dose of tAs in this study was found to be 236.51 µg kg^{-1} day^{-1} (for age group >16) which is higher than reported in all of the earlier studies of Pakistan i.e. 0 to 5.56 x 10^{-4} µg $kg^{-1}day^{-1}$ (Muhammad et al., 2010), 0.11 to 3.7 µg $kg^{-1}day^{-1}$ (Farooqi et al., 2007), 0.29 to 1.43 µg $kg^{-1}day^{-1}$ (Memon et al., 2016), 0.036 to 5.6 µg $kg^{-1}day^{-1}$ (Shakoor et al., 2015), 0.5 to 23 µg $kg^{-1}day^{-1}$ (Rasool et al., 2015). This highest average daily dose of tAs is attributed to the higher geogenic arsenic concentration detected in the ground water sources. Exposure data from this study is also expected to be higher than those reported for other areas of the world such as 2.1 to 4.3 µg $kg^{-1}day^{-1}$ (Nguyen et al., 2009) and 1 µg $kg^{-1}day^{-1}$ (Huy et al., 2014) in Vietnam; 0.023 to 0.0521 µg $kg^{-1}day^{-1}$ in Turkey (Caylak, 2012); 4.5 µg $kg^{-1}day^{-1}$ (Valberg et al., 1997), 2.2 to 3.3 µg $kg^{-1}day^{-1}$ (Meacher et al., 2002) and 177 µg $kg^{-1}day^{-1}$ (Steinmaus et al., 2003) in USA; 73.9 µg $kg^{-1}day^{-1}$ in India (Mazumder et al., 1998); 1.97 to 2.44 µg $kg^{-1}day^{-1}$ in rural Bangladesh (Khan et al., 2009). Most of these studies have used the USEPA default body weight of 70 Kg and water intake of 2 litres per day (US Environmental Protection Agency, 1989). Average daily dose determined in this study was found to be lower than those reported in Bangladesh as 50 to 500 µg $kg^{-1}day^{-1}$ (Karim, 2000) with a body weight of 44 to 55 kg and a water intake of 2.37 to 3.89 litres per day, daily arsenic intake of 1060 µg $kg^{-1}day^{-1}$ (Pokkamthanam et al., 2011) in India with 4 litres per day water intake. Arsenic occurrence in the ground water of Bangladesh i.e. 4227 µg L^{-1} (Chakraborti et al., 2010) was reported far above the Bangladesh drinking water standard of 50 µg L^{-1}. In addition to such an excessive levels of arsenic in water sources, water intake values may also have influenced the higher average daily dose as explained in Rasheed et al. (2017).

There are no set regulatory limits and reference dose (RfD) of organic arsenic species to compare the results, however, a very low concentration of organic arsenic species (below MDLs) have also resulted in very low average daily doses of MMA, DMA and AsB (Table 5.4). Comparing these findings with minimal risk levels (MRLs) defined by the Agency for Toxic Substances and

Disease Registry (Agency for Toxic Substances and Disease Registry, 2007a) has indicated the lower daily intake dose of MMA and DMA.

5.3.4 Ratio between average daily dose (ADD) and reference dose

The reference dose (RfD) is the daily chemical dose that results in no long-term harmful health effects from prolonged exposure (Lee et al., 2005). For water, the regulatory limits are set on the basis of iAs (i.e. RfD: 0.0003 mg $kg^{-1}day^{-1}$) rather than individual arsenic species. The ratio of average daily dose (ADD) to USEPA reference dose (RfD) has resulted in higher chronic non-cancer risk compared to the ratio between ADD and PTDI also set as HQ for tAs as given in Table-5.5.

HQ calculations for AsV have indicated results closer to tAs due to the existence of tAs mainly as AsV and using a similar level of estimated RfD. A HQ for AsIII was determined using the RfD for iAs (0.0003 mg $kg^{-1}day^{-1}$) and was found to be less than 1 for most of the study participants. However, with an estimated RfD (0.000006 mg $kg^{-1}day^{-1}$) based on reported relative toxicity magnitude, a higher level of HQ was depicted (Table-5.5). The difference of possible health risks estimation subjected to daily reference dose or estimated reference doses presses the need to set the regulatory limits for daily intake level of tAs and arsenic species.

This has also been shown in the Food Standards Australia New Zealand (Food Standards Australia New Zealand, 2002), where PTDI of 0.003 mg $kg^{-1}day^{-1}$bw has been recommended and it is higher by 50% than the JECFA/WHO PTDI of 2.1 µg day^{-1} kg^{-1} body weight for iAs. Various levels of HQ as shown below in Table-5.6 have indicated that 95% of 398 persons living in surveyed houses are at risk of a chronic daily intake of arsenic, whereas this intake is expected mainly in the form of AsV (92% of residents with HQ>1) as shown below in Table-5.6.

Table-5.5: Mean Hazard Quotient (HQ) calculated using standard and estimated reference doses at 95% CI

Age groups	Mean Hazard Quotient (HQ) at 95% CI				
	tAs		AsV	AsIII	
	ADD/RfD	ADD/PTDI	ADD/RfD	ADD/RfD	ADD/est. RfD
	RfD for total arsenic: 0.0003 (mg $kg^{-1}day^{-1}$)	PTDI: 2.1 (µg day^{-1} kg^{-1} body weight)*	RfD equivalent to total arsenic: 0.0003 (mg $kg^{-1}day^{-1}$)	RfD for total arsenic: 0.0003 (mg $kg^{-1}day^{-1}$)	est. RfD 0.000006 (mg $kg^{-1}day^{-1}$)
Age 3-6	27.07 (9.95, 44.18)	3.87 (1.42, 6.31)	27.89 (10.23, 45.55)	0.20 (0.14, 0.27)	10.07 (6.85, 13.28)
Age 6-16	52.33 (17.98, 86.68)	7.48 (2.57, 12.38)	54.07 (17.70, 90.45)	0.29 (0.17, 0.42)	14.69 (8.55, 20.83)
Age ≤ 16	50.42 (18.62, 82.20)	7.20 (2.66, 11.74)	52.09 (18.43, 85.75)	0.29 (0.17, 0.40)	14.34 (8.66, 20.03)
Male >16	47.98 (31.50, 62.14)	6.69 (4.50, 8.88)	49.08 (32.05, 66.12)	1.08 (0.62, 1.54)	53.85 (30.88, 76.82)
Female >16	47.26 (25.25, 70.72)	6.85 (3.61, 10.10)	52.15 (26.53, 77.77)	0.56 (0.17, 0.95)	28.09 (8.71, 47.47)
Age>16	47.26 (34.45, 60.08)	6.75 (4.92, 8.58)	50.25 (35.91, 64.59)	0.88 (0.56, 1.20)	44.08 (28.00, 60.15)

*0.0021 mg kg^{-1} day^{-1} body weight

Table-5.6: Results for the chronic exposure assessment

Arsenic species	RfD	Unit	HQ<1 (No effect)		HQ 1-10 (Effect)		HQ >10 (Significant effect)		Overall HQ >1 (Effect)	
			n	%	N	%	n	%	n	%
tAs	0.0003	mg $kg^{-1}day^{-1}$	20	5	181	45	197	49	378	95
	2.1(PTDI)	µg day^{-1} kg^{-1} body weight	146	37	210	53	42	11	252	63
AsV	0.0003	mg $kg^{-1}day^{-1}$	30	8	185	47	183	46	368	92
AsIII	0.0003	mg $kg^{-1}day^{-1}$	362	91	25	6	11	3	36	9
	0.000006	mg $kg^{-1}day^{-1}$	0	0	291	73	107	27	398	100

The average daily intake of arsenic from drinking local domestic ground water in the study area is considerably higher than the levels reported to cause adverse health effects in the scientific literature. Chronic and acute health threats to the exposed rural communities are likely based on the dataset collected here. This is indicated as chronic and acute health complications such as black foot disease at a daily intake of 10 to 50 µg $kg^{-1}day^{-1}$ bw (Agency for Toxic Substances and Disease Registry, 2007b), skin lesions, cardiac or kidney diseases, skin, lung, bladder, respiratory and other types of cancer at dose range of 10 to 40 µg $kg^{-1}day^{-1}$ bw (Lasky et al., 2004; Lubin et al., 2000; Kurttio et al., 1999; Chiou et al., 1995; Hsueh et al., 1995). Furthermore, the latency time between the onset *of* exposure and the appearance of chronic disease endpoints like cancer is reported to be 15 to 30 years depending on daily arsenic intake dose (Agency for Toxic Substances and Disease Registry, 2007b). As such, the study area seems to be a high risk area where household ground water sources (hand pumps and wells) have never been tested for detailed arsenic species. There were general observations of arsenic associated skin problems in the villages Badarpur, Basti Balochan, Chak-46, Chak-48 and Chak-49 observed by the field sampling team with support of medical staff of basic health units. The skin manifestations like hyperpigmentation or hyperkeratosis probably associated with the chronic intake of AsV by the local residents were identified later following the guidelines of the UNICEF clinical diagnostic manual (Sun et al., 2004).

Very high arsenic concentrations found in groundwater might lead to other arsenic related health implications in the near future, if villagers continued to consume arsenic contaminated water and remedial measures are not taken. To provide the rural communities with arsenic free water for drinking and food preparation requires identification of alternative safe water sources and/or selection of arsenic treatment options capable of removing all the arsenic species. Arsenic free sources include surface water and rain water. Arsenic removal options based on oxidation, sedimentation, coagulation, flocculation, sorption and membrane filtration have been developed and adopted in several

arsenic affected regions including Pakistan. Considering the economics, scalability and sustainability aspects, an overview of such technologies (Appendix 5.4) has revealed that most of these options can remove AsV (Ahmed, 2006) but AsIII is comparatively more difficult to remove. AsIII, when present can be oxidized to AsV for efficient removal in household or community level technologies as reported by Lan (2015); Litter et al. (2010); Ramos et al. (2009); Garrido et al. (2008); Ghurye and Clifford (2001); and Pal (2001). Studying arsenic speciation in drinking water sources is critical to understanding potential health risks and geochemical control is needed as an efficient water treatment solution. Understanding the contribution of individual arsenic sources to overall arsenic burden is important in developing the most appropriate risk management strategies.

5.4 Conclusions

Most studies evaluating human exposure to arsenic have focused on total arsenic and the role of individual arsenic species is still a pressing research need. Thus, this is the first study in Pakistan to characterise both the inorganic and organic arsenic species using ion chromatography inductively coupled plasma collision reaction cell mass spectrometry. The highest level of total arsenic in groundwater was found to be 3090 µg L^{-1} and is likely to be the most common pathway for long-term arsenic exposure. AsV was the dominant inorganic arsenic species in 94% of samples across all the villages studied. Nevertheless, AsIII was identified in one village as the dominant pollutant, indicative of a reducing environment in the aquifer, and is considered the most toxic species as well as being difficult to remove using most of the arsenic remediation technologies. Organic arsenic species such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB) were below detection limits, confirming that contamination of aquifers by human impacts (e.g. by use of arsenical pesticides and fertilizer) is low and the predominant source is geological arsenic release. An average daily intake of arsenic up to 236.51 µg kg^{-1} day^{-1} was determined which is the highest of all reported levels in Pakistan and of

several other arsenic affected countries, other than Bangladesh and India. This level of arsenic intake is likely to be associated with potential health risks among exposed rural communities consuming ground water with arsenic above 10 µg L^{-1}. These results may prove useful for risk assessment and for regulatory agencies to reconsider the maximum contaminant level of arsenic in drinking water and define the regulatory limits for arsenic species. Further research efforts are needed to understand the spatial variation of arsenic species in various geological settings and their long term exposure assessment. The study findings also demand the adoption of efficient and sustainable remediation approaches to address the treatment of arsenite (AsIII) for the supply of arsenic free water to rural households.

5.5 References

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Chapter 6: Arsenic species in wheat, raw and cooked rice: exposure and associated health implications

Rasheed H; Kay P**;** Slack R; Gong YY. 2018. Arsenic species in wheat, raw and cooked rice: exposure and associated health implications. *Science of the total Environment.* https://doi.org/10.1016/j.scitotenv.2018.03.339

Abstract

Arsenic concentrations above 10 µg L^{-1} were previously found in 89% of ground water sources in six villages of Pakistan. The present study has ascertained the health risks associated with exposure to total arsenic (tAs) and its species in most frequently consumed foods. Inorganic arsenic (iAs) concentrations were found to be 92.5±41.88 µg kg^{-1}, 79.21±76.42 µg kg^{-1}, and 116.38±51.38 µg kg^{-1} for raw rice, cooked rice and wheat respectively. The mean tAs concentrations were 47.47±30.72 µg kg^{-1}, 71.65±74.7 µg kg^{-1}, 105±61.47 µg kg^{-1}. Wheat is therefore demonstrated to be a significant source of arsenic exposure. Dimethylarsinic acid was the main organic species detected in rice, whilst monomethylarsonic acid was only found at trace levels. Total daily intake of iAs exceeded the provisional tolerable daily intake of 2.1 µg kg^{-1} day^{-1} body weight in 74% of study participants due to concurrent intake from water (94%), wheat (5%) and raw rice (1%). A significant association between tAs in cooked rice and cooking water resulted in tAs intake 43% higher in cooked rice compared to raw rice. The study suggests that arsenic intake from food, particularly from wheat consumption, holds particular significance where iAs is relatively low in water. Chronic health risks were found to be significantly higher from wheat intake than rice, whilst the risk in terms of acute effects was below the USEPA's limit of 1.0. Children were at significantly higher health risk than adults due to iAs exposure from rice and/or wheat. The dietary exposure of participants to tAs was attributable to staple food intake with ground water iAs <10 µg L^{-1}, however the

preliminary advisory level (200 µg kg^{-1}) was achievable with rice consumption of ≤200 g day^{-1} and compliance with ≤10 µg L^{-1} iAs in drinking water. Although the daily iAs intake from food was lower than total water intake, the potential health risk from exposure to arsenic and its species still exists and requires exposure control measures.

6.1 Introduction

Arsenic (As), a naturally occurring metalloid, is widely present as an environmental contaminant and enters the food chain mainly from contaminated water (European Food Safety Agency, 2009) and several widely consumed foodstuffs (Feldmann and Krupp, 2011; Jiang et al., 2015). Seafood has been identified as the main source of organic arsenic (e.g., arsenobetaine and arsenosugars) and is believed to be non-toxic (Taylor et al., 2017; International Agency for Research on Cancer, 2012b). Most exposure and toxicological assessments have focused on inorganic arsenic (iAs) in drinking water. It is yet not fully understood whether exposure to arsenic via most frequently consumed food (e.g. rice and wheat) causes the same health implications as exposure through drinking water.

Exposure from rice has been assessed in a number of studies (U.S. Food and Drug Administration, 2016; Sand et al., 2015; Chen et al., 2016; Davis et al., 2017; Sun et al., 2012). These studies indicate that rice is the most common exposure source for food stuffs. Rice crops have a comparatively higher tendency to take up iAs as they are grown in submerged soil conditions. Among populations not exposed to iAs via drinking water, rice contributes significantly to the iAs intake (Davis et al., 2017).

Wheat is also an important staple food with a worldwide consumption of 730.9 million tonnes, greater than the 506.5 million tonnes of rice consumed annually (Food and Agriculture Organization, 2017). Past studies have reported lower arsenic levels in wheat than rice (Williams et al., 2007b; Su et al., 2010; Bhattacharya et al., 2010) and provided an impetus to further investigate the health risks due to consumption of wheat grown in arsenic affected regions.

Inorganic arsenic is a recognized carcinogen and its chronic exposure has been reported to result in increased risk of bladder, lung, and skin cancer, type 2 diabetes, and cardiovascular disease (International Agency for Research on Cancer, 2012b). Organic arsenic compounds are considered less toxic than iAs but should still be included in exposure assessments. Since toxicity depends on the chemical forms of arsenic, arsenic speciation in rice and wheat can provide useful information for risk assessment and management. The Joint Food and Agriculture Organization and the World Health Organization (FAO/WHO) Expert Committee on Food Additives has set, in 2014, advisory levels of 200 µg kg^{-1} iAs in polished rice grains (Codex Alimentarius Commission, 2014). Apart from the EU regulations (EU) 2015/1006) (European Commission, 2015) on adopting this limit, several countries have still not implemented this limit and are in the process of setting regulatory limits for rice based products. Adoption of this advisory limit in different geographical regions requires exposure assessment via rice. Considering these facts, this study has determined the concentrations of total arsenic (tAs) and As species in wheat, raw and cooked rice to assess the relative contribution of dietary arsenic to aggregate daily exposure. Human health hazards associated with daily consumption of rice, wheat and household groundwater by children (age ≤16 years) and adults (age >16 years) was calculated based on these exposures to provide an indication of hazard of each exposure source.

6.2 Materials and Methods

6.2.1 Study area and study participants

The study villages were located within four districts of Pakistan (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan), where arsenic concentrations above 10 µg L^{-1} were previously found in 89% of household ground water sources. The sampling frame consisted of 223 households comprising 398 volunteers enrolled and interviewed in our previous studies aimed to assess household ground water arsenic concentrations (Rasheed et al., 2017a) and dietary consumption patterns (Rasheed et al., 2017b). Thus, data on age (3-

80 years, mean 36±17 years), gender (246 men and 149 women), body weight (56.6±19.9 Kg), occupation (n=186 farmers and agriculture labour), cooked rice (469 ± 202 g day^{-1} $person^{-1}$) and wheat intake (372 ± 119 g day^{-1} $person^{-1}$) were obtained by questionnaire from 398 participants in the 223 households enrolled in our earlier study (Rasheed et al., 2017b). The households ground water sources (n=228) used both for the drinking and food preparation were found to have mean iAs concentration (204.59 ± 522.88 µg L^{-1}) and associated daily total water intake of 15.401±40.213 g day^{-1} (Rasheed et al., 2017a).

From the same households, only frequently consumed food (wheat and rice) were sampled for this exposure assessment. Raw rice samples were provided by 105 households of villages (Chak-46/12-L, Chak-48/12-I and Chak 49/12-l, Badarpur, Basti Balochan and Kotla Arab), while cooked rice samples could be obtained from 24 households. 12 households provided paired rice samples (raw and cooked both). The main occupation in the study villages was farming with 47% of 398 study participants engaged in this work (Rasheed et al., 2017b), thus, the wheat crop was cultivated and consumed locally within the study villages. Following the sampling strategy of Cubadda et al. (2010), wheat grain samples (n = 189) from two of the most cultivated wheat varieties were collected from the households of six villages. Individual samples (150 g each) were pooled into 8 composite samples weighing in the range of 0.9-7.5 kg.

6.2.2 Samples collection procedure

For raw rice and wheat samples, sterile re-sealable airtight polyethylene zip lock bags were used, whereas for cooked rice (100 grams) 2 oz polyethylene sterile containers were used. After collection, raw rice (250 grams) and wheat samples (150 grams) were stored at room temperature, while cooked rice samples were kept in an insulated cooler containing ice in the field and later stored at -20 °C. Cooked rice samples were shipped to Brooks Applied laboratory, USA by FedEx courier with dry ice under strict quarantine regulations and stored at -20 °C prior to analyses. Raw rice and wheat samples were shipped and stored at ambient temperature (20°C) until

analysis in National water quality laboratory Pakistan and Brooks Applied laboratory (BAL), USA.

6.2.3 Treatment of rice and wheat samples for total arsenic

Rice and wheat samples were rinsed with deionized water (DIW) to remove dust and then dried by air flow at room temperature. Dried samples were milled to powder in a pre-cleaned commercial blender with stainless steel blades. Following USEPA method 3050b (United States Environmental Protection Agency, 1996), a representative 1-2 gram (wet weight) or 1 gram (dry weight) sample was digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The resultant digest was reduced in volume while heating at 95°C ± 5°C and then diluted with ultrapure water to a final volume of 100 mL and subjected to analysis.

6.2.4 Treatment of rice and wheat samples for arsenic speciation

Microwave-assisted HNO_3 digestion for arsenic speciation involved adding 0.35 g of ground raw or cooked rice and wheat samples separately into 15 ml sample tubes. 10 ml of 0.16 M suprapure HNO_3 was added to the tube and left to stand overnight. Microwave irradiation was performed with the temperature profile as: 3 min ramp to 55 °C, 10 min at 55 °C, 2 min ramp to 75 °C, 10 min at 75 °C, 2 min ramp to 95 °C, 30 min at 95 °C. The extracts were centrifuged (10 min, 8000 rpm, 4 °C) and the supernatants filtered through a 0.22 µm filter. The filtrate was stored at 4 °C and analyzed within 24 hours to minimize any species inter-conversion. For final analysis, 0.1 mL of the filtered solution was combined with 0.9 mL of DIW in a 1.5 mL vial and mixed for 10 seconds with a vortex mixer (D'Amato et al., 2011; Raab et al., 2009b).

6.2.5 Analytical procedures

The tAs concentration was measured using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) on an ELAN DRC II ICPMS (Perkin Elmer SCIEX, Concord, Ontario, Canada). Following the methods of D'Amato et al. (2011) and Alava et al. (2012), all sample extracts

were analyzed for iAs (defined as the sum of arsenate (AsV) and arsenite (AsIII)), MMA, and DMA employing an Agilent 7700 CRC ICP-MS with a Dionex GP40 HPLC (IC) System. An aliquot of filtered sample was injected using Dionex HPLC onto an anion-exchange column and mobilized isocratically using an alkaline (pH >7) eluent. The mass-to-charge ratio (m/z) of As at mass 75 was monitored using an Agilent 7700 and the area under the arsenic peaks was used for quantitation. Selenium at m/z 82 was monitored as an internal standard. Retention times for each eluting species were compared to known standards for species identification.

6.2.6 Quality Assurance

For quality control, method blanks, blank spikes, standard reference materials (SRMs) and duplicates were treated in the same way as the samples and incorporated into each digestion batch and analytical run. SRMs include NIST Rice flour (SRM 1568a) for cooked and uncooked rice, NIST Wheat flour (SRM 1567a), and Human hair SRM (NCS DC 73347 from China National Analysis Centre for Iron and Steel Beijing, China) for both hair and nail samples. Data quality in terms of precision, accuracy, method reporting limits (MRLs) and method detection limits (MDLs) met the criteria established in BAL's quality assurance project plan, i.e. relative percent difference (RPD) of <25%, percent recovery of 75 to 125%.

6.2.7 Arsenic Exposure Assessment

Daily intake of tAs and As species for wheat and rice was calculated using Eq. (6.1) (Agency for Toxic Substances and Disease Registry, 2005).

$$EDI = \frac{C \times IR}{BW} \qquad (Eq.6.1)$$

EDI is the estimated daily intake (µg day^{-1} body weight), C represents the average arsenic concentration of rice or wheat (µg g^{-1}), IR is the rice or wheat intake rate (g day^{-1}), and BW is the body weight (kg) of the study individuals. EDI is calculated on the basis of previously published body weights, IR of rice and wheat (Rasheed et al., 2017b), wheat and rice tAs and arsenic species

measured in this study. Raw rice intake was derived from cooked rice by applying a raw-to-cooked rice equivalence factor (Bae et al., 2002).

Total water intake already includes direct drinking water and indirect water intake through food such as cooked rice, wheat bread/chappati, pulses, vegetables, milk, yoghurt and chicken (Rasheed et al., 2017b). Therefore, raw rice intake of iAs instead of cooked rice was taken into account for exposure and risk assessment. EDI values were compared with the World Health Organization's (WHO) provisional tolerable daily intake (PTDI) of 2.1 µg kg^{-1} day^{-1} (World Health Organization, 1989) to assess exceedance. Since the PTDI of 2.1 µg kg^{-1} bw day^{-1} was withdrawn by JECFA in 2010, the ratio between EDI and minimum risk levels set by ATSDR for iAs (Agency for Toxic Substances and Disease Registry, 2017) were calculated for each study participant using Eq. (6,2 and 6.3).

$$\mathrm{HQ} = EDI/MRL_{chronic} \quad \textit{(Eq. 6.2)}$$

$$\mathrm{HQ} = EDI/MRL_{acute} \quad \textit{(Eq. 6.3)}$$

Where;

HQ	Hazard quotient
EDI	Estimated daily intake
MRL	Minimum risk level (chronic exposure 0.0003 mg $kg^{-1}day^{-1}$, acute exposure 0.005 mg $kg^{-1}day^{-1}$) (Agency for Toxic Substances and Disease Registry, 2017)

The Hazard index (HI) was calculated as total non-cancer health hazard posed by iAs through combined daily intake of raw rice and wheat grains using Eq. (6.4 & 6.5) (United States Environmental Protection Agency, 1989).

$$\mathrm{HI} = EDI_{raw\ rice+wheat}/MRL_{chronic} \quad \textit{(Eq. 6.4)}$$

$$\mathrm{HI} = EDI_{raw\ rice+wheat}/MRL_{acute} \quad \textit{(Eq. 6.5)}$$

A calculated HQ or HI greater than 1 suggests that there may be health concerns (United States Environmental Protection Agency, 1989).

6.2.7.1 Evaluation of margins of safety (MoS) for iAs in rice

The Current Codex Alimentarius (CCA), or 'food code' was set in 2014 and sets an advisory level of 200 µg kg^{-1} of iAs in white rice (Codex Alimentarius Commission, 2014), although this limit is still debated and the process of setting legal standards for iAs in rice or rice based products is still incomplete. Modification of the formula used by Shibata et al. (2016) in Eq. (6.6), integrating input variables from this study, was used to assess the suitability of CCA's advisory limit for adoption by regulatory agencies in arsenic affected regions.

$$MTL_{\,rice} = \left(\sum_{3}^{80}((MRL\,.\;BW - (PGV_{water}\,.IR_{water} + C_{wheat}\,.IR_{wheat})).IR_{rice}^{-1}\right).398^{-1} \qquad (Eq.6.6)$$

MTL_{rice} is the maximum tolerable levels of rice, MRL is the minimum risk level defined by Agency for Toxic Substances and Disease Registry (2017) as 0.005 mg $kg^{-1}day^{-1}$ for acute and 0.0003 mg $kg^{-1}day^{-1}$ for chronic arsenic exposure, PGV_{water} is the WHO's Provisional Guideline Value for arsenic (0.010 mg L^{-1} or 10 µg L^{-1}) in drinking water, and IR is abbreviated for the daily intake for water, wheat or rice and C_{wheat} wheat iAs concentration (Table 6.1).

6.2.8 Statistical Analysis

Microsoft Excel and SPSS 24.0 (IBM, New York, NY, USA) were used for statistical analyses. Descriptive analysis was performed for As test data, EDI and HQ of wheat, raw and cooked rice to determine the mean±SD. The data was subjected to bivariate analysis using correlation (Pearson) analysis between different variables to understand their interrelationships. ANOVA was used to test for differences in arsenic between different subgroups with respect to age. A statistical significance level of $p \leq 0.05$ was used.

6.3 Results & Discussion

The present study estimated the arsenic content of wheat, raw and cooked rice grains and the associated health risk posed by exposure to arsenic and its species in the human population of rural settings in Pakistan and data so obtained has been presented and discussed in subsequent sections.

6.3.1 Arsenic speciation and quality control

Mean tAs measured in SRM NIST rice flour (SRM 1568a for cooked and uncooked rice) was 270±10 µg kg^{-1} (n=4), within the certified range of 285 ± 14 µg kg^{-1}, yielding a recovery of 97%. tAs concentration 5.60 µg kg^{-1} measured in SRM NIST wheat flour (1567a) (n=2) was found within the certified range of 4.8 ± 0.3 As µg kg^{-1} yielding a mean recovery of 83%. As no SRM with certified values of arsenic species was available, therefore SRM 1568a was used for quality control in speciation analysis for both rice and wheat. The results indicated 104 ± 1 µg kg^{-1} of iAs (certified value 92 ± 10 µg kg^{-1}), 179.5 ± 0.5 µg kg^{-1} of DMA (certified value 180 ± 12 µg kg^{-1}), 14.5 ± 0.5 of MMA µg kg^{-1} (certified value 11.6 ± 3.5 µg kg^{-1}) and yielded recoveries of 97%, 100% and 75% respectively. These results were also in agreement with earlier reported results of arsenic species in SRM 1568a as 80-110 µg kg^{-1} (iAs), 160-174 µg kg^{-1} (DMA) and 2-14 µg kg^{-1} (MMA) (D'Amato et al., 2011; Carbonell-Barrachina et al., 2012; Antoni, 2016). Overall, the spike recoveries of tAs, iAs, DMA and MMA in digests of matrix spikes (n=3), matrix spike duplicate (n=3), duplicate (n=3), blank spikes (n=3), post spikes (n=3) were 83-93% for wheat and 86-102% for raw and cooked rice.

6.3.2 Arsenic in raw and cooked rice

The mean concentration of tAs in raw rice (47.47±30.72 µg kg^{-1}) was found to be lower than in cooked rice i.e**.** 71.65±74.71 µg kg^{-1} (Table 6.1).

Table-6.1: Summary statistics of As and its species concentrations in raw rice, cooked rice and wheat (µg kg^{-1}) on wet weight basis

Analyte	Statistics	Raw Rice	Cooked Rice*	Wheat
		n=105 (for tAs)	n=24	n=8

		n=10 (for As species)		
tAs	Mean±SD	47.47±30.72	71.65±74.7	105±61.47
	min-max	<LOD-186	24-270	49-241
iAs	Mean±SD	92.5±41.9	79.21±76.42	116.38±51.38
	min-max	63-200	18-300	64-228
DMA	Mean±SD	13±7.38	8.72±13.75	≤LOD
	min-max	LOD-23	≤LOD-48	≤LOD
MMA	Mean±SD	≤LOD	≤LOD	≤LOD
	min-max	≤LOD	≤LOD	≤LOD
Organic As (DMA+MMA)	Mean±SD	13.5±7.38	9.23±13.75	1±0.0
	min-max	1-23.5	1-48.5	1-1
SumAs	Mean±SD	106±47	88.44±82.91	117.38±51.38
	min-max	66.02-223.5	19-309.5	65-229
iAs percentage	Mean±SD	87.53±6.38	91.13±8.78	99.02±0.36
	min-max	80-98.53	69.9-99.67	98.46-99.56
As percentage (orgranic)	Mean±SD	12.47±6.38	8.87±8.78	0.98±0.36
	min-max	1.47-2	0.33-30.1	0.44-1.54

SD: Standard deviation, n= number of samples
LOD: 5 µg kg^{-1} for tAs and 0.5 µg kg^{-1} for iAs, DMA and MMA
* Cooked rice MMA of 83.0 µg kg^{-1} excluded as a single outlier as they exceeded other samples by more than ten times, and, inclusion in data set, would result in twice the current reported mean for the whole sub-group.

The mean tAs concentration in raw rice (n=105) was lower than (108-383 µg kg^{-1}) reported in white polished rice grown in Bangladesh, India, China, Taiwan, Thailand, Vietnam, Spain, Brazil, Turkey and USA (Table 6.2). Our results were higher than the mean tAs of 30-40 µg kg^{-1} for rice grown in Malawi (Joy et al., 2017) and Egypt (Meharg et al., 2009) and comparable to the findings of Rahman et al. (2009) reporting tAs concentrations of 61 µg kg^{-1} in Pakistani Basmati rice available in Australian supermarkets.

The mean tAs concentration of 71.6 µg kg^{-1} (24-270 µg kg^{-1}) in cooked rice (n=24) was lower than mean concentrations (170-370 µg kg^{-1}) previously reported for cooked rice consumed in Bangladesh and West Bengal (Mondal and Polya, 2008; Rahman et al., 2006; Smith et al., 2006; Bae et al., 2002; Roychowdhury et al., 2002). The maximum concentrations in cooked rice were 270 µg kg^{-1} (tAs), 300 µg kg^{-1} (iAs), 48 µg kg^{-1} (DMA), whilst MMAs were detected in raw or cooked rice as ≤LOD.

Table-6.2: Comparison of arsenic and its species in raw polished white rice (µg kg^{-1}) with past studies

Sampling location	n	tAs	iAs	MMA	DMA	Reference
Bangladesh	11	131 (30–300)	83 (10–210)		19 (0–50)	Williams et al. (2005)
India	15	46 (30–50)	27 (20–40)	0.7	66	Williams et al. (2005)
India	29	283 ± 13	194	2.0±0.000	14 ±1.0	Halder et al. (2014)
China	248	116.5	90.9	-	-	Huang et al. (2013)
China	33	230 (19–586)	154 (71–386)	1.3 (7–13)	40 (9-147)	Zhu et al. (2008)
Spain	39	188 ± 78	114 ± 46	-	-	Torres-Escribano et al. (2008)
Spain	7	170	80	<LOD	50	Williams et al. (2005)
Turkey	50	202	159.7	2.7	40	Sofuoglu et al. (2014)
Pakistan	10	47.47 (0.5-186)*	92.50 (63-200)	0.5	13 (0.5-23)	This study
Taiwan	nd	383 (190–760)	247 (110–510)	32 (15–60)	37 (30–50)	Williams et al. (2005)
Korea	30	135	85	20	30	Kim et al. (2013)
Thailand	79	139.48 ± 5.94	81.58	<2.0 (<2.0–6.40)	29.00 (2.42–85.95)	Nookabkaew et al. (2013)
Vietnam	12	136.31 ± 11.42	91.20	<2.0 (<2.0–4.14)	16.25 (5.94–25.08)	Nookabkaew et al. (2013)
USA	24	265 (162–383)	103 (52-217)	0.6(0–6)	155 (40–302)	Zavala et al. (2008)
USA	34	108	65	3	40	Kim et al. (2013)
Brazilian	44	222.9	112 (56-218)	8 (0–29)	93 (39–258)	Batista et al. (2011)

*n=105

The mean iAs of 92.50±41.88 µg kg^{-1} in raw rice and 79.21±76.42 µg kg^{-1} in all cooked rice samples (Table 6.1) revealed only one raw rice (200 µg kg^{-1}) and two cooked rice (290 µg kg^{-1}, 300 µg kg^{-1}) samples which exceeded the preliminary advisory limit of 200 µg kg^{-1} iAs in rice (Codex Alimentarius Commission, 2014). Rice distributed in several areas of Pakistan is mainly produced in the primary rice growing region of Punjab (Rasheed et al., 2016) using ground water and/or surface water irrigation. However, even with low As in irrigation water, rice can accumulate 10-fold higher iAs than other grains (Davis et al., 2017) and may require exposure control measures.

In line with the earlier studies (Williams et al., 2005; Ma et al., 2016; Mondal and Polya, 2008; Rahman et al., 2011), arsenic concentrations in raw rice comprised of >80% of iAs, whilst cooked rice was found to have 69-100% of iAs (Table 6.1). The mean DMA concentration in raw rice (13±7.38 µg kg^{-1}) was higher than in cooked rice (8.72±13.75 µg kg^{-1}) and comparable to the raw rice of south Asian origin, but much lower than rice grown in Brazil and USA (Table 6.2). The higher proportion of iAs and stronger linear relationship with tAs (R^2 = 0.97) than DMA (R^2 = 0.4) has categorized raw rice into “iAs type” as per criteria set by Zavala et al. (2008), whereas, demethylation of DMA and MMA in rice also increase the iAs contents as reported by Chavez-Capilla et al. (2016). Proportion of iAs in raw rice varies geographically depending on the crop variety and uptake of iAs and other arsenic species by crop plants from soil and irrigation water (Santra et al., 2013; Fu et al., 2014; Phan et al., 2014; Talukder et al., 2012). This suggests that arsenic absorption in cooked rice varies with the arsenic concentration in cooking water and with cooking method.

6.3.2.1 Impact of cooking

The tAs concentration in paired raw and cooked rice samples (*n* = 12) was found to be 8-186 µg kg^{-1} (mean 83.1 µg kg^{-1}) in the raw samples and 26-260 µg kg^{-1} (mean 55.29 µg kg^{-1}) in cooked rice respectively (Figure 6.1).

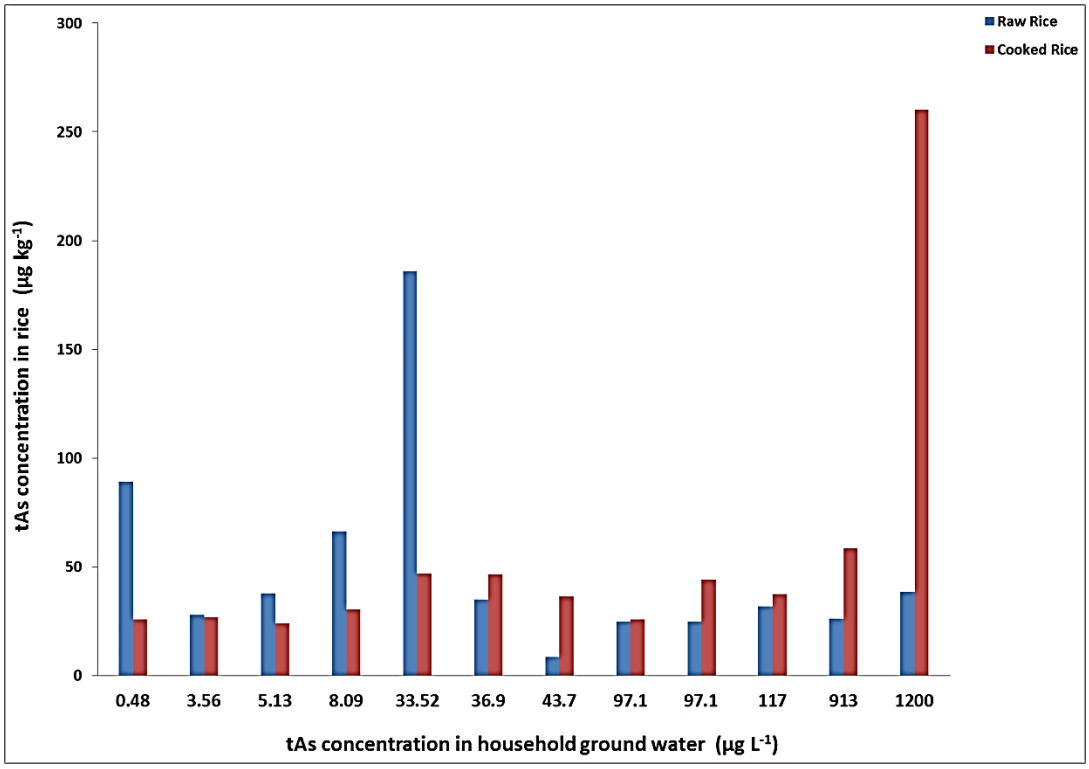

Figure-6.1: The concentration of tAs in raw and corresponding cooked rice samples (n=12)

A significant association (r=0.85, p<0.001) was found between tAs in cooked rice (n=24, mean 71.65 µg kg^{-1}) and tAs of corresponding cooking water (n=24, mean 382.56 µg kg^{-1}). Seven households out of twelve showed an increase of up to 43% in tAs of rice after cooking (Figure 6.1). The five households which cooked in low arsenic water (0.48-33.52 µg L^{-1}) showed a significant decrease of up to 48% (r=0.92, p=0.02) in tAs. An increased tAs in cooked rice is in agreement with Ohno et al. (2009) (raw 220±110 *vs* cooked 260±150 µg kg^{-1}), whilst reduced tAs after cooking in low arsenic water is comparable to other studies (Rahman et al., 2011; Sengupta et al., 2006; Raab et al., 2009a) which showed up to a 57% decrease in cooked rice. As per information inquired from householders, two main cooking methods were used; the Traditional method (A) and the Intermediate method (B) categorized by Signes et al. (2008) but the impact of cooking method on arsenic concentrations in rice requires further investigation.

6.3.3 Arsenic in wheat grains

The mean tAs concentration of 105±61.47 µg kg^{-1} in wheat grains grown in the study area was higher than the mean tAs concentration of 47.47±30.72 µg kg^{-1} in raw rice (Table 6.1). Wheat is grown locally in this study area for household consumption using mainly ground water irrigation, whilst rice is also purchased from local shops indicating the supply of rice from sources beyond the study area. Rice has a greater capacity for As uptake from soil water than wheat. (Williams et al., 2007b; Norra et al., 2005). In this study, higher levels of As in wheat suggests a direct relationship to the use of highly As contaminated irrigation water and it is likely that if rice were grown in this area, As levels in rice might have been higher due to the relatively greater uptake capacity of rice compared to wheat.

The mean tAs concentration in locally cultivated wheat grains (Table 6.1) was higher than the range of 20-129 µg kg^{-1} found in wheat grown in the USA, Netherlands, and India (Gartrell et al., 1986; Wiersma et al., 1986; Sharma et al., 2016; Bhattacharya et al., 2010) but lower than the wheat grown (362 µg kg^{-1}) in West Bengal, India (Roychowdhury et al., 2002). The maximum tAs concentration (241 µg kg^{-1}) was lower than that found in Cornwall, Southwest England (500 µg kg^{-1}) (Williams et al., 2007a) and 317-400 µg kg^{-1} in Pakistan (Baig et al., 2011; Arain et al., 2009). Arsenic determined in wheat was mainly iAs with mean and maximum concentrations of 116.38±51.38 µg kg^{-1} and 228 µg kg^{-1} respectively.

Milling of wheat grains to separate bran from wheat flour may result in a 23−29% reduction of tAs (Zhao et al., 2010). By applying this factor to this study, the mean tAs concentration of wheat grains might be reduced from 105 µg kg^{-1} to 75-81 µg kg^{-1} after milling. However, wheat flour conventionally kneaded in the study area (for chapatti/bread making) with arsenic rich water combined with its high levels of consumption is expected to result in high levels of arsenic exposure.

6.3.4 Estimated daily intake of arsenic from dietary sources

A significantly higher iAs intake from raw rice (0.3±0.1 µg kg^{-1} bw day^{-1}) or cooked rice (0.8±0.4 µg kg^{-1} bw day^{-1}) was found for the 6-16 age group compared to the 3-6 years and >16 years, whilst exposure from wheat intake was significantly higher among children of 3-6 years than other age groups (Table 6.3). The cooked rice iAs exposure for children (<16 years) is comparable to the mean exposure of 0.7 µg day^{-1} for children of 1-2 years old reported by Mantha et al. (2017) and 1-6 years by Yost et al. (2004). Mean iAs exposure from raw rice (0.3±0.1 µg kg^{-1} bw day^{-1}) was higher than for an average 70 kg body weight person in the US (0.02 µg kg^{-1} bw day^{-1}) as reported by Mantha et al. (2017). The mean total daily intake (TDI) of iAs (16±40 µg kg^{-1} bw day^{-1}) comprised 1.5% from raw rice, 4.5% from wheat and 94% from water which was higher than the mean iAs dietary intake (0.1 to 0.4 µg kg^{-1} bw day^{-1}) of the European population (European Food Safety, 2014). Contrary to this study, a maximum cooked rice contribution of 41% was reported by Signes et al. (2008b), suggesting the significance of inter-individual and geographical variations in food safety regulations.

Mean iAs exposure from raw rice (0.3±0.1 µg kg^{-1} bw day^{-1}) was comparable (Jorhem et al., 2008) which showed a rice contribution in Sweden of 1.3% of the provisional weekly tolerable intake (PWTI) of 15 µg kg^{-1} bw (2.1 µg kg^{-1} bw day^{-1}). When compared with the provisional tolerable daily intake (PTDI) of 2.1 µg kg^{-1} bw day^{-1}, 2%, 0.3%, 65% and 74% of the study participants exceeded for iAs intake from cooked rice, wheat, water and TDI respectively (Table 6.3). These finding suggest that the estimated daily intake of iAs from raw rice, cooked rice and wheat grains contributed to a much lesser extent in arsenic exposure, compared to intake from water.

Table-6.3 Descriptive statistics for the body weight adjusted estimated exposures of iAs stratified by study population

Source	Age groups	n	Consumption (g day^{-1})	iAs intake µg kg^{-1} bw day^{-1} (Mean ± SD)	iAs intake µg kg^{-1} bw day^{-1} (Min-max)	% in total dietary intake	n(%) >2.1 µg kg^{-1} bw day^{-1}
Raw Rice	All participants	168	136	0.3±0.1	0.1-0.6	8.2	0
	3-6 years	4	27	0.2±0.1	0.1-0.3	4.1	0
	6-16 years	34	79	0.3±0.1	0.1-0.6	6.4	0
	>16 years	130	154	0.2±0.1	0.1-0.5	8.8	0
	P-value		0.0005	0.033			
Cooked Rice	All participants	168	469	0.7±0.3	0.1-2.4	n.i	2 (1.5)
	3-6 years	4	91	0.7±0.1	0.4-0.7	n.i	0
	6-16 years	34	272	0.8±0.4	0.3-1.7	n.i	0
	>16 years	130	532	0.7±0.3	0.1-2.4	n.i	2 (1.5)
	P-value		0.0005	0.033			
Wheat	All participants	394	372	0.7±0.3	0.2-2.1	21.5	1(0.3)
	3-6 years	4	149	1.1±0.3	0.8-1.5	14.5	0
	6-16 years	59	227	0.9±0.3	0.4-1.7	18.3	0
	>16 years	331	400	0.7±0.3	0.2-2.1	22.1	1(0.3)
	P-value		0.0005	0.0005			
Water**	All participants	398	3.5	15±40	0.02-263	75.3	255 (63)
	3-6 years	5	1.9	8±6	2.6-17	85.1	5 (100)
	6-16 years	61	2.9	16 ±44	0.07-227	78.7	48 (78.7)
	>16 years	332	3.6	15 ±40	0.02-263	74.5	202 (60.8)
	P-value		0.0005	0.126			
Total dietary intake*	All participants	398		16±40	0.4-264		294 (73.9)
	3-6 years	5		10±6	2.8-18		5 (100)
	6-16 years	61		17±44	1-228		54 (88.5)
	>16 years	332		16±40	0.4-264		235 (70.8)
	P-value			0.132			

n.i: not included,; *Based on raw rice, wheat and total water intake

**iAs intake (based on sum of concentrations of AsIII and AsV) from water obtained from our previous study (Rasheed et al., 2017a)

Study participants exposed to iAs (water) <1 µg L^{-1} showed a TDI of 0.5±0.3 µg tAs kg^{-1} bw day^{-1} with approximately 92% of intake from staple food (raw rice and wheat), whereas participants exposed to iAs (water) <10 µg L^{-1} showed a TDI of 0.9±0.3 tAs µg kg^{-1} bw day^{-1} with approximately 60% of intake from food (raw rice and wheat). Study participants exposed to iAs (water) >10 µg L^{-1} had a tAs TDI of 17±39µg kg^{-1} bw day^{-1}, with 4.4% of intake from staple food. These results suggest that the persistent exposure from food should always be taken into account with water for any type of health risk assessment or risk management.

6.3.5 Ratio between combined iAs intake and recommended reference levels

Mean iAs HQ due to chronic exposure from wheat (2.4±1.1) was found to be significantly higher (P=0.0005) than mean HQ for both raw rice consumption (0.8±0.3) and the mean HQ for cooked rice (2.3±1.1) (Table 6.4). These values were found to be higher than the USEPA advised minimal threshold level of 1.00 (United States Environmental Protection Agency, 1989) in 14% (raw rice), 97% (wheat), 94% (cooked rice) of study participants (Table 6.4).

Children of age 3-6 and 6-16 years were the most vulnerable groups compared to adults with HQ>1 due to iAs exposure from wheat, raw rice or cooked rice suggesting an increased risk potency probably due to body weight and water/food intakes differences (Table 6.4). Rice cooked in arsenic rich water (0.48-1270 µg L^{-1}) resulted in higher HQ values in 94% of participants compared to raw rice (14%) and consequently a higher non-cancer health risk (Table 6.4). Mean HI (2.7±1.1) due to concurrent intake of raw rice and wheat grains (without taking water into account) was found to be higher than the safe limit of 1.0, indicating a moderate health risk in 100% of residents (Table 6.4). The risk calculated for acute exposure from all exposure sources showed almost no risk.

Table-6.4: A summary of exposure risks posed to study population due to iAs intake from rice and wheat grains

Age Group (years)	Statistics	HQ (RR)	HQ (Wheat)	HQ (Cooked Rice)	HI

3-6	n	4	4	4	5
	Mean ± SD	0.8±0.1	3.7±1.0	1.9±0.5	3.6±1.7
	n(%) >1	0	4 (100)	4 (100)	4 (80)
6-16	n	34	59	34	61
	Mean ± SD	0.98±0.4	2.9±1.1	2.7±1.2	3.3±1.2
	n(%) >1	15 (44)	59 (100)	33 (97)	60 (99)
>16	n	130	331	130	332
	Mean ± SD	0.8±0.2	2.3±1.1	2.2±1.0	2.6±1.0
	n(%) >1	9 (7)	320 (97)	121 (93)	331 (100)
All participants	n	168	394	168	398
	Mean ± SD	0.8±0.3	2.4±1.1	2.3±1.1	2.7±1.1
	n(%) >1	24 (14)	383 (97)	158 (94)	395 (100)
P-value between age subgroups)		0.033	0.0005	0.033	0.006

RR: raw rice

Study area participants were also eating other food like pulses, vegetables, milk, yoghurt and chicken (Rasheed et al., 2017b) which may also be of concern, but potentially not as great as the concern regarding consumption of staples rice and wheat. Therefore, the exposure data for rice and wheat provided here may prove helpful for regulation of arsenic exposure from the most frequently consumed food. An evaluation of margins of safety for iAs in rice has resulted in the MTL_{rice} of 0.1 mg kg^{-1} due to an average rice consumption of 469 g day^{-1}. The CCA's advisory level of 0.2 mg kg^{-1} iAs in white polished rice is only achievable in a study population with an average rice consumption of 200 g day^{-1} and compliance with 10 µg L^{-1} iAs in drinking/cooking water.

Since As intake from water used for preparation of tea, yoghurt drink (lassi), milk, wheat flour kneading, washing and cooking of rice, chicken, pulses and vegetables (as indirect water intake:Rasheed et al. (2017b)) was taken into account for this exposure assessment, however the future investigation should also consider arsenic speciation of poultry products, locally grown vegetables, and dairy products such as milk, butter and meat of livestock reared with arsenic contaminated water.

6.4 Conclusions

Inorganic arsenic exposure from consumption of wheat was higher in this study population than rice followed by lower levels of dimethylarsinic acid (DMA) from raw and cooked rice. Raw rice was a moderate source of exposure in the study

villages although cooking in arsenic rich, low volumes of cooking water, and higher cooked rice consumption frequency may contribute significantly in producing a potential risk. The prolonged arsenic exposure of study participants from total water intake (including indirect water used for rice cooking and wheat flour kneading), raw rice and locally grown wheat, was demonstrated by a total daily intake of 16±40 µg iAs kg^{-1} bw day^{-1} with relative contributions from food (6%), drinking and cooking water (94%). The chronic non-cancer risks due to aggregated exposure of iAs from wheat and raw rice have indicated somewhat higher mean hazard quotient values (2.7±1.1) than the acceptable limit of 1.0 in 100% of participants. Children were subject to significantly higher exposure and health risks compared to adults**.** Dietary exposure to inorganic arsenic occurs naturally such as in raw rice or wheat grains and is unavoidable; however growing the crops with low arsenic irrigation water, rice cooking and wheat flour kneading in low arsenic water may reduce the dietary exposure. The study findings suggest that an inorganic arsenic maximum tolerable level for the most frequently consumed food such as rice and wheat as well as recommendations on their consumption frequency would be useful to lower the exposure risk. Moreover, arsenic remediation of water used for drinking, irrigation and food preparation is an immediate requirement for populations in arsenic affected regions.

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Chapter 7: Assessment of arsenic species in human hair, toenail and urine and their association with water and staple food

Rasheed H; Kay P; Slack R; Gong YY. Chapter 7: Assessment of arsenic species in human hair, toenail and urine and their association with water and staple food (forthcoming in Nature Journal: *Exposure Science and Environmental Epidemiology*)

Abstract

Arsenic intake from household drinking/cooking water and food may represent a significant exposure pathway to induce cancer and non-cancer health effects. This study has shown the relative contribution of water and staple food to arsenic intake and accumulation by multiple biological matrix measurements of inorganic and organic arsenic species, while accounting for potential confounders such as age, gender, occupation, and exposure duration. Multivariate linear regression showed a strong significant relationship between total arsenic (tAs) intake from water and concentrations of tAs, inorganic arsenic (iAs), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) in urine and toenail samples. tAs intake from staple food (rice and wheat) also showed a strong significant relationship with hair tAs and iAs. The sole impact of staple food intake on biomarkers was assessed and a significant correlation found with all of the urinary arsenic metabolites. Toenail was found to be the most valuable biomarker of past exposure to inorganic and organic arsenic species of dietary and metabolic origin.

7.1 Introduction

Human exposure to toxic inorganic arsenic (iAs) via water is a recognized public health and scientific concern (Cottingham et al., 2013). Recently detected arsenic concentrations in food have also raised the question as to the contribution from food. Based on evidence of carcinogenicity in humans, the International Agency for Research on Cancer (IARC) classified arsenic and iAs compounds as ‘carcinogenic to humans’ (Group 1) and classified dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) as ‘possibly carcinogenic to humans’ (Group 2B)

(International Agency for Research on Cancer, 2012). A sequence of reduction and methylation reactions in the human body metabolises iAs into monomethylarsonic acid (MMA), which is further methylated to DMA (Aposhian and Aposhian, 2006, Orloff et al., 2009). Following ingestion, iAs compounds are well-absorbed by humans at an estimated rate of 50 and 95% (Agency for Toxic Substances and Disease Registry, 2007).

Most of the ingested arsenic is excreted as methylated arsenic within 1-3 days following exposure although a part of it is stored in sulphydryl-rich tissue such as skin, nail and hair (Raab and Feldmann, 2005). Average per day growth rates for fingernails (0.1 mm), toenails (0.1 and 0.03–0.5 mm) and hair (0.2 to 1.12 mm) depict exposure during the last 6, 12–18 and 3-12 months, respectively (Hinwood et al., 2003; Fleckman and Allan, 2001; Garland et al., 1993). This makes nail and hair effective biomarkers of past exposure, however arsenic toxicokinetics depend on the forms of arsenic and variations in association with various factors such as age, sex, nutritional status and genetic polymorphisms (European Food Safety, 2014). Types and levels of excreted methylated arsenic as a useful biomarker may vary with such factors although few studies have assessed their impact (Ahsan et al., 2006; Lindberg et al., 2008; Shen et al., 2016; Tsuji et al., 2004). Arsenic speciation in hair toenail/nail has been inadequately performed, whilst the association of arsenic intake from water and food with inorganic and organic arsenic species in hair, toenail and urine has also been insufficiently studied. Thus, the objectives of this research were set to (1) assess human exposure to As through measurement of total arsenic (tAs) and arsenic species in hair, toenail, and urine, and; (2) study the impact of dietary exposure (including water) on the internal dose of arsenic species in relation to potential modifiers.

7.2 Materials and Methods

7.2.1 Study area and study participants

The study villages were located within four districts of Pakistan (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan), where at least one ground water source was found to be contaminated with arsenic above 50 µg L^{-1}. The sampling frame consisted of 398 volunteers (223 households in six villages) enrolled and

interviewed in our previous studies aimed to assess household ground water arsenic concentrations (Rasheed et al., 2017a) and dietary consumption patterns (Rasheed et al., 2017b). Residents of these villages were mostly dependent on the household ground water sources (wells, hand pumps) installed 8 to 44 years ago and previously found to have tAs of 0.48 to 3090.00 µg L^{-1} (Rasheed et al., 2017a). The participants were non-smoking males and females who used their household ground water for drinking and food preparation did not eat seafood, use any homeopathic or herbal medicines and were not away from their houses for more than a week during the sampling months of August-October, 2014 for collection of urine, hair and toenail samples. Pregnant women were excluded from the study and after all exclusions, urine (n=395), toenail (n=20) and hair (n=19) samples were collected.

7.2.2 Collection of urine, hair and toenail samples

Spot urine samples from 246 males and 149 females were collected in labelled sterile 2 oz polyethylene urine collection containers and kept in an ice box at 4 °C prior to return to the laboratory. All urine samples were transferred to a field freezer within 2 hours for storage at −20 °C and transported to the National Water Quality Laboratory, where creatinine was determined on a 1 mL sub-sample. All samples were then shipped with dry ice to the Brooks Applied Laboratory (BAL), USA by air, stored at −70 °C, and finally measured for urinary arsenic metabolites within 4 months.

Using ethanol-rinsed stainless-steel scissors, a full strand of hair sample was obtained by the sampling team from the nape of the head as near as possible to the scalp (at a distance of 1 cm from scalp). Hair samples were stapled on cardboard, placed in sealed plastic bags and stored at room temperature until analysis. Participants were asked to remove nail polish, if any, and collect their toenail clippings from all toes using the provided stainless steel clippers (Hinwood et al., 2003; American Industrial Hygiene Association, 2004). These were placed in individual polyethylene bags, shipped to BAL and stored at ambient temperature (20°C) until analysis.

7.2.3 Urine samples processing and analysis

Urinary concentrations were corrected for creatinine concentrations, which were determined by the Jaffe method as described by Bonsnes and Hertha (Bonsnes and Taussky, 1945). This correction was done by dividing the concentration of arsenic metabolites (μg L^{-1}) by U-Cre (g L^{-1}) to express urinary arsenical species as μg g^{-1} creatinine.

Frozen urine samples were thawed to room temperature and centrifuged at 3000 rpm for 10 min and the resultant supernatants were diluted 10-fold with ultrapure water and analyzed for tAs following U.S. Environmental Protection Agency method 1638 (mod.) using inductively coupled-plasma dynamic reaction cell-mass spectrometry (Model: ELAN DRC II ICPMS, Perkin Elmer SCIEX, Concord, Ontario, Canada). For measurement of urinary arsenic species i.e. arsenate (AsV), arsenite (AsIII), MMA, DMA and arsenobetaine (AsB), aqueous samples were filtered through a 0.45-μm filter. The filtered aliquot were analysed by high-performance liquid chromatography system (Dionex GP-40) coupled to an inductively coupled plasma – mass spectrometer (ICP-MS) (Agilent 7700x ICPMS, Agilent Technologies) following the method described by Hata (2007). Urine samples after processing were rapidly analysed to ensure appropriate preservation of organic species. Since As(III) can oxidize to As(V) (Agency for Toxic Substances and Disease Registry, 2007) during samples handling and laboratory processing, thus urinary iAs was presented as the sum of As(III) and As(V). The limits of detection were 0.1 μg L^{-1} for tAs, As(III), DMA, and AsB, 0.3 μg L^{-1} for As(V) and 0.2 μg L^{-1} for MMA.

7.2.4 Hair and toenail samples processing and analysis

Each hair sample was cut to a length of 0.125-inch (0.3-cm), representing approximately the last two months of As exposure before sampling. Past studies evaluating the external contamination of hair and nail have reported that washing procedures effectively removed the exogenous As from toenail and hair samples (Middleton et al., 2016b; Button et al., 2009b). Thus, external contamination from hair and toenail clipping samples was removed by immersing samples three times in 5 ml of a 0.5% Triton TX-100 solution and shaking thoroughly by hand for 30 seconds. Samples were rinsed three times with 18.2 MΩ deionised water (DIW) and then twice with HPLC grade acetone (Button et al., 2009b). Hair samples

underwent the same cleaning and digestion procedure as toenail samples. Polycarbonate filters (0.4 µm) and an anti-static device were used for the transfer of hair samples between vessels. Following rinsing, samples were dried overnight at room temperature and weighed. Following USEPA method 3050b (United States Environmental Protection Agency, 1996), an aliquot of dried toenail or hair sample was prepared by adding multiple additions of HNO_3 and hydrogen peroxide (H_2O_2) and heating at 95 ˚C ± 5 ˚C. After cooling, the volume was made up to 100 mL with DIW, centrifuged and stored at room temperature until analysed exclusively for endogenous arsenic and its species. Total arsenic was measured using the technique of inductively coupled-plasma dynamic reaction cell-mass spectrometry (Model: ELAN DRC II ICPMS, Perkin Elmer, Shelton, CT, USA). All sample extracts for arsenate (AsV), arsenite (AsIII), MMAs, and DMAs quantitation were also analyzed employing an Agilent 7700 CRC ICP-MS with a Dionex GP40 HPLC (IC) Systems.

For speciation, an aliquot of filtered sample was injected using a Dionex HPLC onto an anion-exchange column and mobilized isocratically using an alkaline (pH > 7) eluent. The mass-to-charge ratio (m/z) of As at mass 75 was monitored using an Agilent 7700, whilst selenium at m/z 82 was monitored as an internal standard. Retention times for eluting species were compared to NIST traceable known standards for species identification.

7.2.5 Quality assurance

Species data was provided by the analysis of NIST (National Institute of Standards and Technology) traceable standard reference materials (SRMs-1640A, trace elements in natural water). Background contamination was monitored using laboratory fortified blanks for urine analysis. Duplicate measurements were made on 10% ($n = 40$) of urine samples for total arsenic and arsenic species. The reliability of the arsenic species determination was evaluated by analysing samples in duplicate and spiking the samples with As(III), As(V), MMA, DMA and AsB. Arsenic measured in SRMs-1640A was 7.59 ± 0.36 tAs µg kg^{-1} (n = 6), within the certified range of 8.010 ± 0.067 µg kg^{-1}, yielding a mean recovery of 96%. The spike recoveries of tAs, AsIII, AsV, DMA, MMA and AsB in digests of matrix spikes (n=31), matrix spike duplicates, duplicates (n=40) and laboratory fortified blank

(n=6) met the data quality standards in terms of relative percent difference (RPD) of <25%, percent recovery of 75 to 125% and completeness of 80%.

For quality control of hair and nail samples, method blanks, blank spikes, standard reference materials (SRMs) and duplicates were treated in the same way as the samples and incorporated into each digestion batch and analytical run. Human hair SRM (NCS DC 73347 from China National Analysis Centre for Iron and Steel Beijing, China) was used for both hair and nail samples. Arsenic measured in SRM NCS DC 73347 was 274 ± 0.5 tAs μg kg^{-1} (n = 2), within the certified range of 280 ± 50 μg kg^{-1}, yielding a mean recovery of 98%. There is no available SRM of human hair or nail containing certified concentration for arsenic species. The organic species represented a minimum fraction of tAs in SRM NCS DC 73347, whilst iAs was more than 65% of the extraction indicated as the main proportion of As in hair. The spike recoveries of tAs, iAs, DMA and MMA in digests of matrix spikes (n=2), matrix spike duplicate (n=2), duplicate (n=2), blank spikes (n=2), and post spikes (n=2) were 83-92% for hair and 93-123% for toenail.

7.2.6 Statistical analysis

The analysed tAs represents the sum of As species as well as other unidentified forms of As species, whilst the SumAs is defined as the sum of urinary iAs, MMA and DMA. Mass balance was assessed by the difference of tAs intake and tAs excreted assuming the mean 24-h urine volume of 1.5 L day^{-1} (based on urine output of 2.0 L day^{-1} for men and 1.6 L day^{-1} for women given by EFSA, 2010). Urine, toenail and hair As concentrations had positively skewed distributions therefore logarithmic transformations applied for statistical analysis. For this analysis, concentrations below the limit of detection (LOD) of the test methods were replaced by a value equal to half of the LOD.

ANOVA and student's t-test were used to test for differences in natural log transformed values of urine, toenail and hair arsenic concentrations between different subgroups with respect to age (≤16 and >16 years), gender, ground water tAs concentration, occupation and exposure duration. Multiple linear regression models were constructed to assess significant predictors of biomarkers while

controlling for possible confounding factors. The independent variables were log-transformed values of daily As intake from water and staple food (rice and wheat). The dependent variables were log-transformed concentrations of toenail and hair (tAs, iAs, MMA and DMA), and urine (tAs, iAs, MMA, DMA and SumAs). Considered potential confounders were age, gender, occupation and exposure duration. Before multivariate analyses, bivariate analyses (Pearson correlations) were conducted to assess associations between potentially confounding factors and biomarkers. Factors associated with a *P*-value<0.1 were first selected then the factors with the weakest *P*-value were inserted in the multivariate linear regression model using forward selection. The multivariate models were checked for multicolinearity and goodness of fit. Microsoft Excel, SPSS 24.0 (IBM, New York, NY, USA) and GraphPad Prism 7.0 were used for statistical analyses. The statistical significance level of $P \leq 0.05$ was set for the multivariate analysis.

7.3 Results and Discussion

7.3.1 Study population characteristics

Data on the estimated daily total arsenic (tAs) intake from water, rice and wheat was obtained from previously published studies (Rasheed et al., 2017a; Rasheed et al., 2017b) and further, as yet, unpublished work (Table 7.1).

Table-7.1: Selected characteristics of study participants who provided urine, hair and toenail samples

Characteristics	n	GM (min-max)	Data source
Study participants	398		This study
Urine samples	395		
Hair samples	19		
Toenail samples	20		
Age			Rasheed et al. (2017b)
≤16 years	66		
>16 years	332		
Gender			
Male	249		
Females	149		
Body weight (Kg)	398	52.19 (9-105)	
Exposure duration from ground water tAs (years)		14.7 (3-44)	Rasheed et al. (2017a)
8-13	212		
13-15	62		
15-44	124		

Characteristics	n	GM (min-max)	Data source
tAs concentration in household ground water (µg L^{-1})			
Overall	398		
≤10	50		
10-50	145		
>50	203		
Estimated daily tAs intake (µg kg^{-1} bw day^{-1})			
Drinking/cooking water	398	3.217 (0.02-236.510)	
Participants consumed rice only	4	0.176 (0.122-0.226)	Rasheed et al. (2017b)
Participants consumed wheat only	230	0.609 (0.194-2.234)	
Participants consumed staple food (wheat+rice)	164	0.589 (0.275-2.0235)	
Occupation category			
Labour non-Intensive (n=149)			
House wives (general)	45		
Students	75		
Tailors	4		
Teachers	4		
Un-employed	21		
Labour intensive (n=249)			
Farmers	186		
Wives/family member of farmers (contributing in farming)	56		
Services	7		

GM:Geometric mean

The study participants had an age range of 3–80 years at the time of sampling with 37% female participants and 10% participants above 60 years of age. The household's drinking/cooking water was found to have a GM tAs concentration of 55.33 µg L^{-1} and a range of 0.48-3090 µg L^{-1}, with 89% of sources above the WHO provisional guideline value (10 µg L^{-1}) for arsenic in drinking water (World Health Organization, 1996).

7.3.2 Urinary biomarker levels in relation to population subgroups

The GMs for the concentrations of urinary tAs (234.43 µg g^{-1} creatinine), iAs (26.98 µg g^{-1} creatinine), MMA (23.32 µg g^{-1} creatinine) and DMA (142.80 µg g^{-1} creatinine) for all study participants and for different demographic and behavioural subsets are shown in Tables 7.2.

The DMA metabolite was the predominant form of As in urine (representing 71% of the sum of urinary arsenic metabolites), followed by iAs (13%) and MMA (12%). This conforms to the findings of Melak et al. (2014) indicating As excretion as iAs (10–20%), MMA (10–15%) and DMA (60–75%) depending on inter-individual

variation. AsB generated as a result of seafood ingestion, was not detected in the study population.

The significant impact ($P < 0.001$) of ground water tAs concentration (<10 µg L^{-1}, 10-50 µg L^{-1} and >50 tAs µg L^{-1}) on urinary arsenic metabolites (Table 7.2) was found in concordance with the other studies on low arsenic regions (Middleton et al., 2016a, Button et al., 2009a). There was a significant age-dependent trend for urinary tAs concentrations ($P = 0.032$) whilst males had significantly higher concentrations of urinary tAs, iAs, MMA, SumAs ($P \leq 0.05$) than females. The trend of higher MMA excretion in men than women (27.72 vs. 17.47 µg g^{-1} creatinine) was consistent with previous investigations (Zhang et al., 2014, Nizam et al., 2013). This difference was reported to be linked with choline synthesis under the effect of estrogen in women of childbearing age (Shen et al., 2016; Lindberg et al., 2008). Estrogen contributes to the synthesis of choline by regulating the phosphatidylethanolamine methyltransferase (PEMT) pathway (Vahter, 2007).

Table-7.2: Geometric means [GM (min-max)] for creatinine adjusted urinary arsenic metabolites (µg g^{-1} creatinine)

Characteristics	n	Urine Creatinine	tAs	iAs	DMA	MMA	Sum As
Overall	385	0.99 (0.35-2.55)	234.43 (7.78-8743.59)	26.98 (0.139-1411.11)	142.80 (0.08-2353.53)	23.32 (0.08-615.31)	201.38 (0.30-4375.76)
Age							
≤16 years	62	0.92 (0.56-1.56)	302.38 (27.55-8743.59)	30.44 (0.23-1357.24)	162.99 (0.13-1704.08)	26.52 (0.14-615.31)	230.81 (0.49-3676.63)
>16 years	323	1.02 (0.35-2.55)	223.17 (7.78-3969.70)	26.36 (0.14-1411.11)	139.22 (0.08-2353.54)	22.75 (0.08-611.11)	196.17 (0.30-4375.76)
p-values (t test)		0.03	0.032	0.424	0.411	0.415	0.395
Gender							
male	241	1.03 (0.35-2.55)	267.13 (7.78-8743.59)	30.60 (0.14-1411.11)	158.71 (0.08-2353.54)	27.72 (0.08-611.11)	226.30 (0.30-4375.76)
female	144	0.96 (0.54-2.01)	188.97 (10.30-4510.20)	21.85 (0.23-1357.24)	119.67 (0.11-1955.22)	17.47 (0.14-615.31)	165.65 (0.49-3676.63)
p-values (t test)		0.02	0.002	0.013	0.052	0.001	0.020
tAs in water (µg L^{-1})							
<10	50	0.98 (0.50-1.93)	113.76 (10.297-760.60)	14.53 (1.43-123.03)	87.45 (9.167-488.46)	11.81 (1.38-102.02)	116.75 (12.12-677.58)
10-50	140	0.97 (0.41-2.55)	163.46 (18.636-1233.33)	19.72 (1.29-229.62)	118.15 (9.93-967.68)	16.59 (0.76-251.28)	159.13 (17.80-1220.64)
>50	195	1.02 (0.35-2.45)	360.50 (7.778-8743.59)	39.60 (0.14-1411.11)	185.54 (0.08-2353.54)	35.47 (0.08-615.31)	274.25 (0.30-4375.76)
p-values (ANOVA)		0.350	0.0005	0.0005	0.0005	0.0005	0.0005
Occupation							
Labour intensive	242	1.024 (0.53-2.55)	213.44 (7.78-2563.64)	24.34 (0.14-381.11)	129.06 (0.08-1990.91)	21.16 (0.08-415.45)	182.22 (0.30-2767.36)
Labour non-Intensive	143	0.96 (0.50-2.03)	274.62 (12.62-8743.59)	32.11 (0.23-1411.11)	169.47 (0.13-2353.54)	27.49 (0.14-615.31)	238.49 (0.488-4375.76)
p-values (t test)		0.036	0.019	0.042	0.061	0.067	0.046

*Urine samples for tAs (n=395)

Non-intensive labour occupations were associated with significantly increased tAs, iAs and SumAs concentrations ($P < 0.05$) compared to labour intensive occupations (Tables 7.2). Exposure duration (≤14 and >14 years) did not have a significant impact on urinary concentrations.

Mass balance was estimated to determine which source provided the majority of the tAs intake. Out of tAs intake (842.69 µg day^{-1}) from total water intake water (799.47 µg day^{-1}) and staple food (43.22 µg day^{-1}), the mean tAs excreted in urine was 591.18 µg day^{-1}. The remaining 251.51 µg day^{-1} was assumed to be internally absorbed and/or excreted in faeces. The tAs intake from the consumption of food (43.22 µg day^{-1}) represents only 7.31 % of the excreted tAs.

7.3.3 Toenail and hair biomarkers levels in relation to population subgroups

A significant increase in toenail and hair concentrations of tAs and its species ($P \leq 0.001$) was found with increasing drinking/cooking water tAs concentration (<10 µg L^{-1} to >50 tAs µg L^{-1}) except for hair DMA (Table 7.3). The binding of iAs, dietary and/or metabolically produced DMA and MMA with sulfhydryl nails is reported to be partly dependent on the concentration available in the blood (Grashow et al., 2014). Thus, participants with longer exposure duration (>14 years) had significantly higher concentration of toenail and hair tAs and iAs, indicative of prolonged exposure (Table 7.3). Age and gender did not show a significant impact on toenail and hair concentrations (data not shown). Type of occupation (labour intensive and non-labour professions) showed no impact. Despite the higher outdoor activities of participants engaged in labour intensive occupations (services, farmers, wives of farmers contributing in farming), significantly higher toenail DMA in participants of non-labour intensive occupations (general house wives, students, tailors, teachers and un-employed) was unclear (Table 7.4).

Table-7.3: Geometric means [GM (min-max)] for arsenic and arsenic species in toenail and hair (µg kg^{-1})

Characteristics	n (toenail)	n (hair)	Toenail, GM(min-max) (µg kg^{-1})				Hair, GM(min-max) (µg kg^{-1})			
			tAs	iAs	MMA	DMA	tAs	iAs	MMA	DMA
Overall	20	19	1942.18 (586-27500)	1756.91 (557-22000)	79.44 (6-955)	21.88 (0.8-432)	702.16 (67.0-3100.0)	653.25 (84-10700)	1.43 (0.5-55)	2.64 (0.5-123)
tAs in water (µg L^{-1})										
<10	5	5	593.06 (586-599.2)	568.43 (559.2-578)	32.82 (26-39)	0.91 (0.8-1)	73.82 (67-94.1)	90.06 (84.0-95)	2.46 (2.0-3.0)	3.36 (2.0-7)
10-50	4	3	1321.94 (602-4070)	1217.91 (557-3840)	32.72 (6-97)	12.88 (6-57)	1006.65 (352-2760)	830.73 (325-2250)	0.72 (0.6- 0.9)	10.12 (0.6-69)
>50	11	11	3830.19 (1190-27500)	3352.55 (1270-22000)	163.93 (77-955)	112.52 (25-432)	1771.87 (531-13100)	1505.83 (438-10700)	1.35 (0.5-55)	1.64 (0.5-123)
p-values (ANOVA)	-	-	0.0005	0.001	0.001	0.0005	0.0005	0.0005	0.0005	0.364
Occupation										
Labour intensive	13	12	1766.00 (586-27500)	1627.03 (559-22000)	67.12 (6-955)	11.81 (0.8-432.0)	504.73 (67-13100)	507.16 (84-10700)	1.39 (0.5-17)	1.69 (0.5-69)
Non-Labour intensive	7	7	2317.31 (605.0-4660)	2026.26 (557-4070)	108.65 (48-209)	68.75 (9.1-310.0)	1236.57 (352-4610)	1008.19 (325-3590)	1.52 (0.5-55)	5.66 (0.5-123)
p-values (t test)			0.53	0.59	0.25	0.04	0.190	0.34	0.89	0.28
Exposure duration										
≤14 years	13	12	1277.25 (586.0-4660)	1163.46 (557.0-4070)	66.54 (26.0-209)	13.48 (0.8-310.0)	330.06 (67.0-3770)	329.59 (84.0-3140)	1.57 (0.5-17.0)	1.34 (0.5-7.0)
>14 years	7	7	4229.75 (2060.0-27500)	3777.22 (1840-22000)	110.41 (6.0-955)	53.74 (6.0-432)	2561.18 (615.0-13100)	2110.63 (669.0-0700)	1.23 (0.5-55.0)	8.50 (0.5-123)
p-values	-	-	0.012	0.009	0.331	0.123	0.005	0.004	0.703	0.119

(t test)

Table-7.4. Multivariate linear regression analysis of associations between log transformed values of estimated daily intake of tAs (µg kg^{-1} bw day^{-1}) and exposure biomarkers

Independent variable	Biological Matrix	Biomarkers	β coefficient	Std. Error	p-value	Model Adjusted R^2
tAs intake from drinking water	Urine	tAs	0.307	0.028	0.0005	0.276 [2]
		iAs	0.3	0.038	0.0005	0.168 [2]
		DMA	0.229	0.042	0.0005	0.069 [5]
		MMA	0.284	0.04	0.0005	0.158 [2]
		Sum As	0.259	0.038	0.0005	0.104 [5]
	Toenail	tAs	0.348	0.063	0.0005	0.612 [3]
		iAs	0.342	0.056	0.0005	0.660 [3]
		DMA	0.672	0.08	0.0005	0.606 [5]
		MMA	0.24	0.122	0.008	0.294 [5]
	Hair	tAs	0.443	0.073	0.0005	0.792 [1]
		iAs	0.386	0.07	0.0005	0.764 [1]
		DMA	-0.291	0.159	0.15	0.243 [5]
		MMA	0.009	0.19	0.958	-0.17 [5]
tAs intake from staple diet	Urine	tAs	0.577	0.106	0.0005	0.122 [2]
		iAs	0.894	0.132	0.0005	0.105 [5]
		DMA	0.773	0.143	0.0005	0.068 [5]
		MMA	0.866	0.138	0.0005	0.136 [2]
		Sum As	0.812	0.131	0.0005	0.088 [5]
	Toenail	tAs	1.017	0.291	0.003	0.547 [1]
		iAs	0.995	0.265	0.002	0.587 [1]
		DMA	2.698	0.598	0.0005	0.504 [4]
		MMA	1.131	0.336	0.003	0.352 [5]
	Hair	tAs	1.725	0.357	0.0005	0.718 [1]
		iAs	1.547	0.322	0.0005	0.718 [1]
		DMA	-1.139	0.700	0.128	0.258 [1]
		MMA	0.043	0.591	0.943	-0.169 [5]

[1] adjusted for exposure duration
[2] adjusted for gender and occupation
[3] adjusted for gender
[4] adjusted for occupation
[5] other potential confounders did not contribute significantly to the models were excluded by statistical programme

7.3.4 Intercorrelations among exposure biomarkers

The concentration of urinary iAs was significantly correlated with urinary MMA (r=0.905, $P \leq 0.0001$) and DMA (r=0.884, $P \leq 0.0001$). Whilst, urinary MMA was significantly associated with DMA (r=0.912, $P \leq 0.0001$). Urinary iAs was significantly correlated with toenail tAs (r=0.484, P=0.036), toenail iAs (r=0.494, P=0.031), hair tAs (r=0.513, P=0.030) and hair iAs (r=0.487, P=0.040). A significantly strong association between hair tAs (r=0.779, $P \leq 0.0001$) and toenail (tAs) also exist.

Significant positive intercorrelations between urinary, toenail and hair arsenic species suggest that either of these may be used as biomarkers of arsenic exposure, however these biomarkers reflect the As exposure over different time periods as mentioned in section 7.1.

7.3.5 Multivariate linear regression analysis of relations between tAs intake and exposure biomarkers

Multiple linear regression analysis revealed a positive significant relationship between the tAs intake from drinking/cooking water and urinary tAs, iAs and MMA after adjusting for gender, occupation and exposure durations (Table 7.4). The association between urinary arsenic metabolites and drinking water arsenic concentrations are in line with the results of multiple regression models from previous studies (Normandin et al., 2014; Rivera-Nunez et al., 2012) indicating a positive relation between estimated intake of tAs from drinking water and urinary As species adjusting for gender (Table 7.4).

A significant positive association existed between tAs intake from staple food and those of urinary arsenic metabolite concentrations when adjusted for gender and occupation. The predictor variables such as drinking/cooking water and food tAs intakes both showed significance with response variables i.e. toenail tAs, iAs, MMA, DMA and hair tAs and iAs, indicating the mean change in the response variable for one unit of change in the predictor variable while holding gender, occupation and exposure duration as constant (Table 7.4). The influence of gender, exposure duration and occupation subgroups on urine, hair and toenail tAs and arsenic species suggests the possible underlying reasons. These include metabolic, inter-individual, social-demographic and behavioural variability, growth rate of skin appendages, health status, nutrition or exogenous contamination from dust or soil in crop field and kinetic models for peripheral tissues (Grashow et al., 2014). Study participants exposed to tAs (water) <1 µg L^{-1} and <10 µg L^{-1} showed a staple food tAs intake of 0.485 µg kg^{-1} bw day^{-1} (n=5) and 0.733 µg kg^{-1} bw day^{-1} (n=50) respectively. No significant impact of tAs intake from food was found on urinary arsenic metabolites below 1 µg L^{-1}. However, participants exposed to <10 µg L^{-1} tAs concentration of drinking/cooking water (n=50) showed significant Pearson correlation (P <0.05:

data not shown) between tAs intake from food and urinary arsenic metabolites, suggesting the sole contribution of food in human exposure to arsenic.

The regression model coefficients (Table 7.4) showed that for every additional unit of tAs intake from water, an average increase of urinary tAs by 220.74 µg g^{-1} creatinine (urine), 1944.96 µg kg^{-1} (toenail) and 755 µg kg^{-1} (hair) was expected. Compared to this, tAs intake from food shows increased tAs concentration by an average of 456.23µg g^{-1} creatinine (urine), 5721.58 µg kg^{-1} (toenail) and 4272.70 µg kg^{-1} (hair). This increase due to food tAs intake was higher by an average factor of 3.6 when compared to values derived from model coefficient of water tAs intake. These findings showed that water and food tAs intake were found as the strongest predictors of all of the urinary and toenail biomarker concentrations. When compared to food, drinking/cooking water was a relatively stronger predictor as seen by adjusted R-square values (Table 7.4). Though the sample size of toenail and hair could constitute a limitation of this study, the degree of significant associations revealed that toenail arsenic speciation is a more precise biomarker of effects, a potential determinant of prolonged arsenic exposure and indicative of critical arsenic related health effects. In the same context, an elevated risk of cutaneous melanoma (Beane Freeman et al., 2004) and lung cancer (Heck et al., 2009) was reported in persons with higher toenail arsenic concentrations.

7.4 Conclusions

The consumption of drinking/cooking water containing total arsenic concentrations previously found in household hand pumps/wells of rural settings of Pakistan significantly increased the absorbed dose of tAs, iAs and its mono- and di-methylated arsenic in urine, hair and toenail under the influence of certain biological and behavioural modifiers such as gender, exposure level, occupation and exposure duration. Levels of these species in biological matrices can also increase significantly due to exposure through frequent consumption of staple foods such as rice and wheat. The levels of tAs, iAs and its mono- and di-methylated arsenic in urine, hair and toenail were also influenced by certain biological and behavioural modifiers such as gender, exposure level, occupation

and exposure duration. Association of toenail arsenic with water and food intake of arsenic can be observed as a more favourable biomarker of arsenic exposure than urine and hair.

Given the critical role of highly reactive and genotoxic intermediate trivalent forms of MMA and DMA produced from methylation of inorganic arsenic, this study underscores the need to determine these trivalent forms in association with potentially modifying effects of dietary and occupational exposure along with confounding factors such as smoking, nutrients, genetics, education on arsenic accumulation and excretion.

7.5 References

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Chapter 8: The effect of association between inefficient arsenic methylation capacity and demographic characteristics on the risk of skin lesions

Rasheed H; Kay P; Slack R; Gong YY. 2017. The effect of association between inefficient arsenic methylation capacity and demographic characteristics on the risk of skin lesions. *Toxicology and Applied Pharmacology.*
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Abstract

This study was conducted in rural Pakistan to assess the dose-response relationship between skin lesions and arsenic exposure and their variation by demographic characteristics. The study included 398 participants (66 participants with skin lesions and 332 without) residing in six previously unstudied villages exposed to ground water arsenic in the range of <1 to 3090 µg L^{-1}. The skin lesions identification process involved interview and physical examinations of participants followed by confirmation by a physician according to UNICEF criteria. Urinary inorganic arsenic (iAs), total arsenic (tAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were analysed to determine methylation capacity, methylation efficiency and the dose-response relationship with skin lesions. Study participants with skin lesions were found to be exposed to arsenic >10 µg L^{-1} with a daily arsenic intake of 3.23±3.75 mg day^{-1} from household ground water sources for an exposure duration of 10-20 years. The participants with skin lesions compared to those without skin lesions showed higher levels of urinary iAs (133.40 ± 242.48 *vs.* 44.24 ± 86.48 µg g^{-1} Cr), MMA (106.38 ± 135.04 *vs.* 35.43 ± 39.97 µg g^{-1} Cr), MMA% (15.26 ± 6.31 *vs.*12.11 ± 4.68) and lower levels of DMA% (66.99 ± 13.59 *vs.* 73.39 ± 10.44) and secondary methylation index (SMI) (0.81 ± 0.11 *vs.* 0.86 ± 0.07). Study participants carrying a lower methylation capacity characterized by higher MMA% (OR 5.06, 95% CI: 2.09-12.27), lower DMA% (OR 0.64, 95% CI: 0.33-1.26), primary methylation index (PMI) (OR 0.56, 95% CI: 0.28-1.12) and SMI (OR 0.43, 95% CI: 0.21-0.88) had

a significantly higher risk of skin lesions compared to their corresponding references after adjusting for occupation categories. The findings confirmed that inefficient arsenic methylation capacity was significantly associated with increased skin lesion risks and the effect might be modified by labour intensive occupations.

8.1 Introduction

Arsenic (As) exposure from drinking water has placed about 200 million people worldwide at risk of arsenic induced health hazards (National Research Council, 2001). Epidemiological studies have revealed the associations between arsenic exposure and multiple health effects. These include developmental effects, neurotoxicity, diabetes, pulmonary disease and cardiovascular disease (Agency for Toxic Substances and Disease Registry, 2007). Arsenic is a recognized carcinogen causing cancer of the skin, liver, lung, kidney, prostate and bladder (International Agency for Research on Cancer, 2012; Mendez et al., 2017; Hong et al., 2014). Skin lesions are a typical sign of arsenic toxicity appearing after a persistent arsenic ingestion for 5-10 years (Lien et al., 1999; Guha Mazumder et al., 1998). There is considerable evidence of the prevalence of arsenical skin lesions in Bangladesh (Ahsan et al., 2006), India (Guha Mazumder et al., 1998), Mongolia and China (Sun, 2004).

Inorganic arsenic (iAs) ingested from drinking water is metabolized in the human body first by its methylation to monomethylarsonic acid (MMA) and then to dimethylarsinic acid (DMA), resulting in iAs excretion from the body as MMA and DMA (Vahter, 2002). Earlier studies have revealed the relationship between urinary arsenic metabolites and arsenic induced skin disorders (Lindberg et al., 2008; Kile et al., 2011). However, the individuals within the same region or population may have different urinary arsenic levels and methylation capacity even when exposed to the same level of arsenic (Vahter, 1999). This suggests there may be variable disease susceptibility among the exposed persons within a population. Nevertheless, the associations between inadequate arsenic methylation capacity and arsenic-induced health effects may be further influenced by demographic and socio-economic features, inter-individual

variability, genetic or geographical variations (Chen et al., 2013; Lindberg et al., 2010; Steinmaus et al., 2006).

Earlier studies in Pakistan (Fatmi et al., 2013; Fatmi et al., 2009; Ahmed et al., 2014) have assessed the association between water and/or urinary iAs concentrations and the prevalence of skin lesions. This investigation focused on the influence of urinary arsenic metabolites and arsenic methylation capacity on disease susceptibility which is, as yet, unstudied. The prevalence of arsenic related skin manifestations had not been scientifically investigated in this study population and hence evaluated as a biological marker of individual exposure. Moreover, to address the arsenic mitigation challenges, identifying the risk groups in the population of arsenic affected regions is also required (National Research Council, 2001; Jakariya et al., 2005).

8.2 Methodology

8.2.1 Study Design and population

The present work is a cross-sectional study involving individuals exposed to arsenic from six villages in the districts of Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan, Pakistan. Our previous study showed that drinking water was the primary source of arsenic exposure beyond the WHO provisional guideline value (10 µg L^{-1}) in the selected villages (Rasheed et al., 2017a). Selection of sample size, recruitment of study participants and demographic characteristics have been published elsewhere (Rasheed et al., 2017b). The 398 non-smoking participants recruited had lived in the study villages for the last 5 years and children (<5 years) by birth and provided consent to being interviewed and physically examined. Health care services in these rural settings were not well organized and no systematic patient records were available to track their arsenic related medical history.

8.2.2 Physical examination of skin

Initially, study participants were observed and interviewed at their houses by the trained non-physician health workers to record observations on general health status and to specifically screen the individuals with cutaneous signs of skin lesions. Unlike skin cancer, which takes decades to develop, these lesions can

appear within a few years of exposure and usually progress through stages. The diagnostic guidelines of the UNICEF clinical diagnostic manual (Sun Guifan et al., 2004) were followed in this screening process. The interviewers, unaware of the health status of the participants, interviewed them using a questionnaire (Appendix 8.1) that collected information on general wellbeing and visible skin lesions which were digitally photographed without facial identification. Following the steps indicated in Figure-8.1, initially screened individuals (n=80) were re-examined after a week at the basic health unit (BHU) of each village by a physician with expertise in detection and diagnosis of skin lesions.

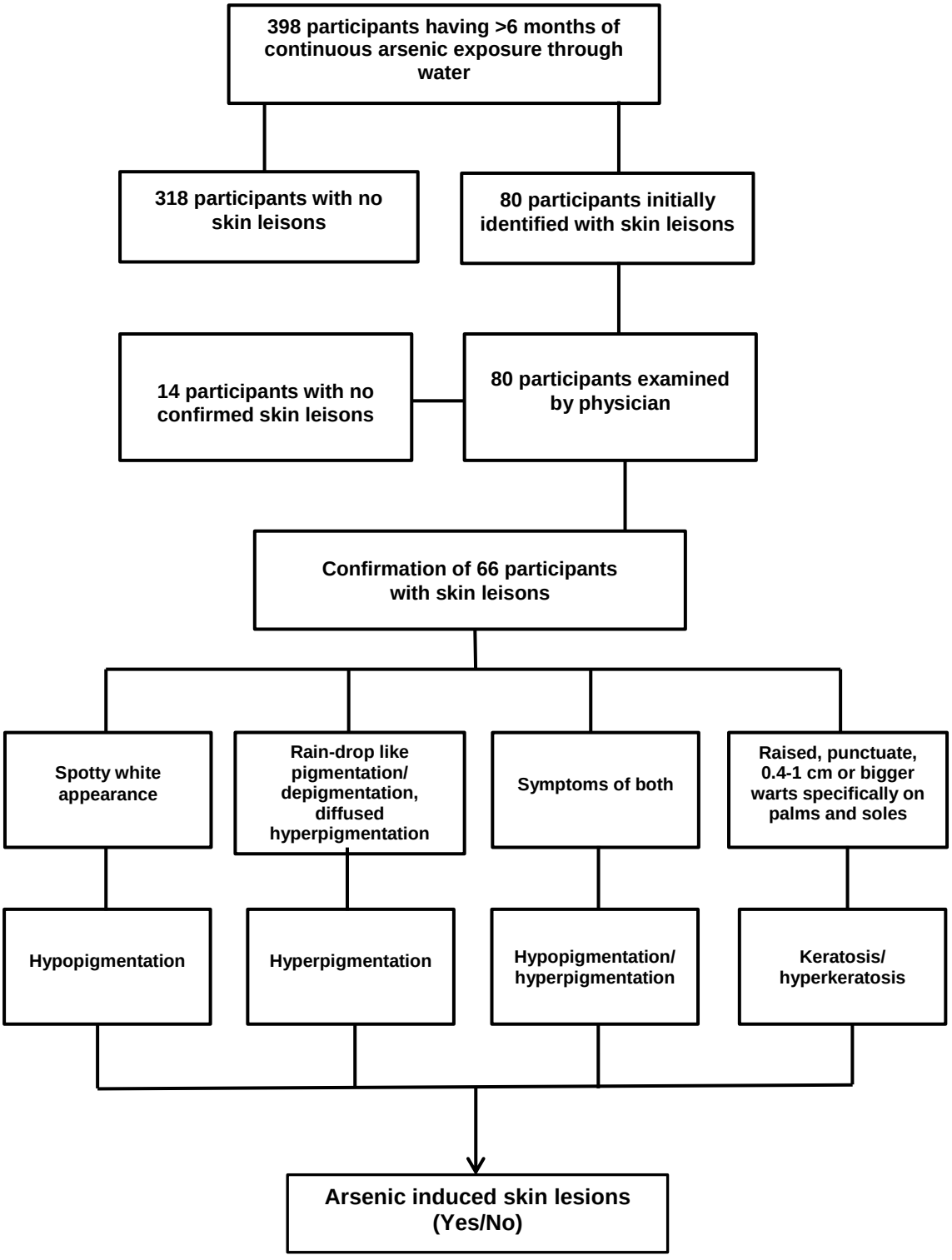

Figure-8.1: Steps involved in screening of participants with arsenic-induced skin leisons

In accordance with the earlier mentioned diagnostic guidelines (Sun Guifan et al., 2004), hyperpigmentation was symptomized as raindrop-like spots, diffused dark

brown spots or darkening of the skin on the limbs or chest, back, and abdomen. Keratosis was identified as thickening of the skin of the palms of hands or the soles of feet, or small flanges (0.4 to 1 cm in diameter) emerged as small corn-like elevations on palms and soles. Initially screened individuals were physically examined to ascertain the presence, shape and location of visible skin lesions. Out of 80 individuals initially screened as patients, 14 cases were confirmed as not having arsenic induced skin lesions. Thus, the study population was grouped into two subgroups including participants with arsenic specific skin lesions (n=66) and those without such skin lesions (n=332).

8.2.3 Measurement of Urinary Arsenic Metabolites

The spot urine samples were collected from all participants in a labelled sterile 2 oz polyethylene urine collection container and kept in an ice box for three hours. Exactly 1 mL of urine was kept separately for creatinine (Cr) determination. All urine samples were then immediately transferred to the National Water Quality Laboratory at −20 °C, where creatinine was determined. All samples were then shipped with dry ice to the Brooks Applied Laboratory (BAL), USA by air and stored at −70 °C, and finally measured for urinary arsenic metabolites within 4 months. Three of the study participants did not provide their urine samples. In total, 395 samples were collected, as well as field duplicates (4% of samples, n=15). Due to spillage during transportation, ten samples did not have enough volume for arsenic speciation. Thus, the Brooks Applied Laboratory (BAL) received 395 samples for total arsenic and 385 samples for arsenic speciation. Urinary creatinine concentration was measured by means of the kinetic Jaffe method using a colorimetric auto-analyzer (Hitachi Ltd., Tokyo, Japan) based on the reaction between creatinine and alkaline picrate (Bonsnes and Taussky, 1945). Concentrations of urinary arsenic species were adjusted using urinary creatinine to correct for variable water excretion rates at the time of specimen collection (Barr et al., 2005). This adjustment was done by dividing the concentration of arsenic metabolites (μg L^{-1}) by U-Cre (g L^{-1}) to express urinary arsenical species as μg g^{-1} creatinine. Frozen urine samples were thawed to room temperature and centrifuged at 3000 rpm for 10 min and the resultant supernatants were used for arsenic analysis. The supernatants were diluted 10-

fold with ultrapure water and analyzed. Total arsenic was measured using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) on a ELAN DRC II ICPMS (Perkin Elmer SCIEX, Concord, Ontario, Canada) following U.S. Environmental Protection Agency method 1638 mod. (U.S. Environmental Protection Agency, 1996). Urinary arsenic speciation i.e. arsenate (AsV), arsenite (AsIII), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB) were measured on an anion-exchange high-performance liquid chromatography system (Dionex GP-40) coupled to an inductively coupled plasma – mass spectrometer (ICP-MS) (Agilent 7700x ICPMS, Agilent Technologies) following the proprietary BAL method. Aqueous samples were filtered through a 0.45-µm filter and an aliquot injected onto an anion-exchange column. Measures used to ensure appropriate preservation of MMA and DMA species in urine samples included sample preservation and preparation at low temperatures, immediate freezing upon collection, least sample treatment before analysis, and rapid speciation when analysed. Whilst As(III) can oxidize to As(V) during sample transport, storage, and preparation, these are expressed as total iAs (i.e. As(III)+As(V). The limits of detection were 0.1 µg L^{-1} for tAs, As(III), DMA, and AsB, 0.3 µg L^{-1} for As(V) and 0.2 µg L^{-1} for MMA.

The proportions of urinary arsenic metabolites (iAs%, MMA% and DMA%) and methylation indices, the primary methylation index (PMI) and secondary methylation index (SMI) were calculated to reflect the arsenic methylation capacity. The arsenic methylation indices were defined as the percentages of iAs%, MMA% and DMA%, calculated by dividing the concentration of each species by the sum of iAs, MMA and DMA. The PMI was calculated as the ratio between MMA+DMA and tAs (Equation-8.1), and the SMI as the ratio between DMA and MMA+DMA (Equation-8.2), (Sun et al., 2007).

$$PMI = \frac{MMA + DMA}{tAs} \qquad (Eq.8.1)$$

$$SMI = \frac{DMA}{(MMA + DMA)} \qquad (Eq.8.2)$$

Quality assurance of urinary arsenic species data was provided by the analysis of NIST (National Institute of Standards and Technology) traceable standard reference materials (SRMs-1640A, trace elements in natural water). Background contamination was monitored using laboratory fortified blanks for urine analysis. Duplicate measurements were made on 10% ($n = 40$) of urine samples for total arsenic and arsenic species (Table-8.1). The reliability of the arsenic species determination was evaluated by analysing samples in duplicate and spiking the samples with AsIII, AsV, MMA, DMA and AsB. Data quality in terms of precision, accuracy, method reporting limits (MRLs), method detection limits (MDLs) and completeness met the criteria established in the BAL's quality assurance project plan (QAPP), i.e. relative percent difference (RPD) of <25%, percent recovery of 75 to 125% and completeness of 80%. Field duplicates for urine indicated mean percentage differences of ≤10% for tAs, MMA and DMA (Appendix 8.2).

8.2.4 Individual exposure assessment

All household ground water samples were collected at the houses of study participants from six selected villages during June-September, 2014 after the skin lesions examinations. These were analysed for total arsenic using USEPA method 200.8 (U.S. Environmental Protection Agency, 2008) and arsenic species by the Brooks Applied Laboratory using ion chromatography inductively coupled plasma collision reaction cell mass spectrometry (BAL proprietary method). These data were published previously (Rasheed et al., 2017a). Daily arsenic intake (mg day^{-1}) was calculated by multiplying the household ground water arsenic concentration (µg L^{-1}) by the daily water intake from the household ground water source (L day^{-1}). Thus, exposure in this study was assessed using urinary arsenic metabolites and tAs of household ground water. In order to reduce the potential bias, the participants and health examiners were unaware of the individual arsenic levels of water samples collected from household ground water sources which were analysed after completion of the survey.

8.2.5 Covariates

In addition to the primary exposure variable we evaluated other covariates suspected to be associated with arsenic exposure. These covariates included socio-demographic factors i.e. age, sex, body weight, exposure duration, daily water intake, villages and occupation, and were derived from the questionnaire based interviews with study participants, published previously in (Rasheed et al., 2017b).

8.2.6 Statistical Analysis

Since the urinary arsenic metabolites data had a positively skewed distribution, natural logarithmic transformations were used to normalize their distributions and the means as well as the 95% confidence interval (CI). Mean arsenic concentrations in urine and household ground water were calculated for participants with and without skin lesions. The Student *t* test and Chi-square test was used to assess the differences of exposure variables between participants with and without skin lesions.

Urinary arsenic metabolites and methylation indices were stratified into quartiles (0-25%, 25-50%, 50-75% and 75-100%) when estimating the odd ratios (ORs) for having skin lesions. Variables measured on a continuous scale, including age, body weight, daily arsenic intake and arsenic exposure, were categorized to evaluate risk. Univariate and multivariate logistic regression analyses were used to evaluate the effect of increasing levels of arsenic intake from water, urinary arsenic metabolites and urinary arsenic methylation indices on the risk of skin lesions. The results of logistic analyses were presented as ORs along with their 95% CIs. Only covariates revealed to be significant in the univariate logistic regression and factors of interest were included in the multivariate regression analysis. We used a p value of <0.05 for statistical significance. Microsoft Excel and SPSS 17.0 (IBM, New York, NY, USA) were used for the statistical analysis.

8.3 Results

8.3.1 Characteristics of the study population

The baseline characteristics of all participants by status of skin lesions are given in Table 8.1.

Table 8.1: The baseline characteristics of the study participants

Characteristics	n	Overall (Mean±SD)	with skin lesions (Mean±SD) (n=66)	Without skin lesions (Mean±SD) (n=332)[a]	p-value
Age of participants (years)	398	35.74±16.99	39.92±15.19	34.91±17.23	0.001***
Body weight (kg)	398	56.66±19.92	64.45±15.43	55.11±20.37	0.0005***
Daily total water intake (L person^{-1} day^{-1})	398	3.47±0.955	3.98±0.97	3.38±0.92	0.0005***
Daily arsenic intake from water (mg day^{-1})	398	0.78±2.01	3.23±3.57	0.32±0.98	0.0005***
tAs conc. in water (µg L^{-1})					
Chak-46/12-L	121	62.28±39.42	113.38 ± 47.82	53.34 ± 30.09	-
Chak-48/12-I	54	275.30±335.97	497.51 ± 433.07	164.17± 204.30	-
Chak 49/12-I	75	54.57±26.18	81.99 ± 13.37	51.75 ± 25.58	-
Basti Balochan	44	24.88±0.68	NA	24.88 ± 6.81	-
Badarpur	34	1605.64±882.51	1874.26± 776.88	1043.98± 854.0	-
Basti Kotla Arab	70	14.784±13.96	NA	14.784 ± 13.95	-
Overall tAs	398	209.08±519.20	828.46±934.28	85.96±245.38	0.0005***
Urinary arsenic Concentration (µg g^{-1} Cr)					
Urinary tAs	395	407.66±659.34	760.48±883.81	336.87±580.81	0.0005***
iAs	395	59.52±131.45	133.40±242.48	44.24±86.48	0.0005***
MMA	385	47.59±71.60	106.38±135.04	35.43±39.97	0.0005***
DMA	385	255.19±301.20	464.70±518.34	211.85±208.90	0.008**
Urinary arsenic proportions and methylation indices					
iAs%	395	15.05±8.99	17.75±9.66	14.50±8.77	0.001***
MMA%	385	12.65±5.13	15.26±6.31	12.11±4.68	0.0005***
DMA%	385	72.29±11.28	66.99±13.59	73.39±10.44	0.006**
PMI	385	0.85±0.09	0.82±0.10	0.86±0.09	0.032*
SMI	385	0.85±0.08	0.81±0.11	0.86±0.07	0.003**

[a]n varies for results of urinary arsenic metabolites and methylation indices.
SD: Standard deviation
p ≤ 0.05* , p ≤ 0.01**, p ≤ 0.001***

The age, body weight, daily water intake, tAs in household water sources and daily water intake were higher among participants with skin lesions than those

without skin lesions. Urinary arsenic metabolites such as tAs, iAs, MMA and DMA were higher in participants with skin lesions than those without skin lesions. AsB, excreted as a result of seafood ingestion, was not detected in this study population. Participants with skin lesions also possessed higher means for urinary iAs%, MMA%, lower urinary DMA% and lower PMI and SMI compared with participants without skin lesions (Table 8.1). The distribution of cutaneous signs observed in the study participants (Figure 8.2) varied; hypopigmentation (9.5%), hyperpigmentation (23.8%), hypo and/or hyperpigmentation (6.3%), melanosis (7.9%), whilst keratosis/hyperkeratosis on the palm or sole was the most prevalent cutaneous sign of arsenicism (47.6%).

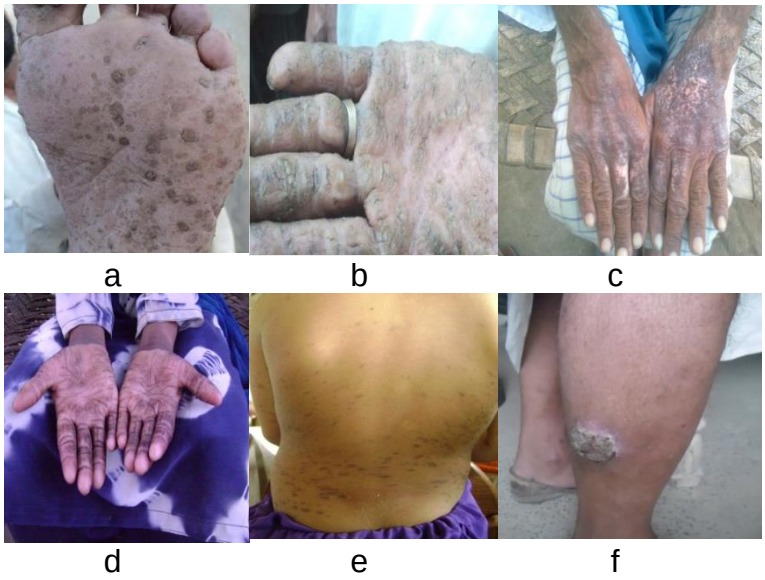

Figure-8.2(a–f): Different types of arsenic-specific skin lesions
(a) Keratosis on sole (b) Keratosis on palm (c) Hypopigmentation on hand
(d) Hyperpigmentation on palms (e) Melanosis on trunk (f) Hyperkeratosis on lower limb.

8.3.2 Association between Urinary Arsenic Methylation Indices and Skin lesions

Table 8.2 shows the distribution of subgroups with and without skin lesions by sex, age, daily arsenic intake, villages, body weight and occupation. Males were more likely than females to have skin lesions (OR 1.90, 95% CI: 1.05-3.45). Compared with the participants in the youngest age group (≤16 years), the risk

of skin lesions increased nearly threefold for participants in the oldest age group >16 years as indicated by an OR of 3.56 (95% CI: 1.25-10.15).

There were no skin problems among participants exposed to ground water tAs levels <10 µg L^{-1}. The association between tAs in water and skin lesion (Table 8.2) showed a significant increasing linear trend from 10-50 µg L^{-1} (OR 1.00: reference) to >50-100 µg L^{-1} (OR 23.4, 95% CI: 3.06-178.68) and >100 µg L^{-1} (OR 219, 95% CI: 29.14-1645.70). Consequently, the OR estimates also increased significantly ($p<0.001$) with increasing arsenic intake (0.001-11.773 mg day^{-1}). Risk was significantly higher for the subgroup in the upper quartile of daily arsenic intake (OR 126, 95% CI: 16.89-939.46) suggesting a dose response effect of arsenic exposure from drinking water intake (Table 8.2). A direct association was found between body weight and skin lesion risk ($p=0.016$), with a threefold increase with increasing body weight >35 kg (OR 3.63, 95% CI 1.273-10.35). Based on the socioeconomic situation, intensity of physical and outdoor activities, and occupations of the study participants they were divided into labour intensive (farmers, wives of farmers and service providers like security guards, drivers etc.) and non-labour intensive subgroups (non-working house wives, students, tailors, teachers and un-employed). The labour intensive category indicated a higher risk of skin lesions (OR 2.83, 95% CI: 1.48-5.39). At village level, a significant increase in the prevalence of skin lesions was found in arsenic affected villages (Table 8.2), with the highest prevalence of 67.7% skin lesion in Badarpur (OR 20.31, 95% CI: 7.04-58.57), where 95.8% of hand pumps were contaminated with arsenic.

ORs for association of urinary arsenic metabolites with the risk of skin lesions using multiple logistic regression analysis after adjustment for confounding factors, such as age, sex, daily arsenic intake, villages, body weight and occupation, were determined. A higher degree of effect was found when adjusting with occupational categories, as presented in Table 8.3. After adjustment for occupation, a significantly higher skin lesion risk was found in the third (OR 6.35, 95% CI: 2.08-19.44; p = 0.001) and fourth quartiles (OR 13.07, 95% CI: 4.30-39.68; p = 0.000) of urinary tAs. A significantly increased risk was found for participants in 4^{th} quartiles of urinary iAs (OR 5.61, 95% CI: 2.48-12.70; p = 0.000)

Similarly, a significantly increased risk was found in the 4th quartile of MMA (OR 5.83, 95% CI: 2.57-13.24; p = 0.000). The 3rd and 4th quartiles of urinary DMA showed significantly higher ORs for skin lesions (Table 8.3). Participants with the highest urinary iAs% (OR 2.65, 95% CI: 1.22-5.75) and MMA% (OR 5.06, 95% CI: 2.09-12.27) showed a significantly highest risk of skin lesions as compared to their reference quartiles (Table 8.4). Participants in the 2nd quartiles (OR 0.64, 95% CI: 0.33-1.26) of urinary DMA% showed a significantly higher risk of skin lesions as compared to their reference quartiles before and after adjustment for villager's occupations. A significant increased risk of skin lesions was detected in participants in the 2nd quartile of PMI (OR 0.56, 95% CI: 0.28-1.12) and SMI (OR 0.43, 95% CI: 0.21-0.88) both before and after adjustment (Table 8.4).

Table 8.2. The ORs for skin lesions by levels of demographic and lifestyle factors

Co-variates		Total number of participants (*n*=398)	Without skin lesion (*n*=332)	With skin lesion (n=66)	Prevalence %	p-value	OR (95% CI)
		n	n	n			
Sex	female	149	132	17	11.4	0.034*	1.00 (ref)
	male	249	200	49	19.7		1.90 (1.05, 3.45)
Age	≤16 years	66	62	4	6	0.018*	1.00 (ref)
	>16 years	332	270	62	18.67		3.56 (1.25,10.152)
tAs in household water sources (µg L^{-1})	10-50	147	146	1	0.68	p<0.001***	1.00 (ref)
	50-100	123	106	17	13.82		23.4 (3.06,178.68)
	>100	80	32	48	60		219.0 (29.142,1645.7)
Daily arsenic intake (mg day^{-1})	Q1:0.001-0.070	99	99	0	0	p<0.001***	-
	Q2:0.071-0.160	100	99	1	1		1.00 (ref)
	Q3:0.162-0.330	99	90	9	9.1		10.01 (1.24,80.59)
	Q4:0.332-11.773	100	44	56	56		126.0 (16.89,939.46)
Villages	Chak 49/12-I	75	68	7	9.3	p<0.001***	1.00 (ref)
	Chak-46/12-L	121	107	18	14.9		1.70 (0.67, 4.28)
	Chak-48/12-I	54	34	18	33.3		4.86 (1.86, 12.71)
	Badarpur	34	12	23	67.7		20.31 (7.04, 58.57)
	Basti Balochan	44	44	0	0		0
	Basti Kotla Arab	70	70	0	0		0
Body weight (kg)	≤ 35 kg	67	63	4	6	0.016*	1.00 (ref)
	> 35 kg	331	269	62	18.7		3.63 (1.273,10.35)
Occupation	Labour non-Intensive	149	136	13	8.7	0.002**	1.00 (ref)
	Labour intensive	249	196	53	21.3		2.83 (1.48,5.39)

CI, Confidence interval
Q: Quartile
p ≤ 0.05* , p ≤ 0.01**, p ≤ 0.001***

Table 8.3. The logistic regression analysis of ORs, unadjusted and adjusted[a], for skin lesions risk by level of urinary arsenic metabolites

Urinary arsenic exposure measures (quartiles)		With skin lesions (n=66)	Without skin lesion (n=332)	Unadjusted OR (95% CI)	p-Value	Adjusted OR[a] (95% CI)	p-value
Urinary tAs (µg g^{-1} Cr) [b]	7.78-123.42	4	94	1.00 (ref)	-	1.00 (ref)	p ≤ 0.001***
	123.58-246.94	11	88	2.94 (0.90-9.57)	0.074	3.14 (0.96-10.31)	0.059
	247.19-426.67	21	78	6.33 (2.08-19.21)	0.001***	6.35 (2.08-19.44)	0.001**
	441.12-8743.59	30	72	9.79 (3.30-29.05)	0.0005***	13.07 (4.30-39.68)	p ≤ 0.001***
Urinary iAs (µg g^{-1} Cr) [b]	0.14-13.796	9	87	1.00 (ref)		1.00 (ref)	p ≤ 0.001***
	13.81-28.58	8	88	0.88 (0.32-2.38)	0.8	1.00 (0.37-2.75)	0.993
	28.66-56.58	14	82	1.65 (0.68-4.02)	0.27	1.81 (0.74-4.47)	0.195
	58.24-1411.11	35	75	4.51(2.04-9.99)	0.0005***	5.61 (2.48-12.70)	p ≤ 0.001***
Urinary MMA (µg g^{-1} Cr) [c]	0.08-10.89	9	87	1.00 (ref)		1.00 (ref)	p ≤ 0.001***
	10.9-27.03	6	90	0.64 (0.22-1.89)	0.423	0.76 (0.26-2.24)	0.617
	27.32-54.44	16	80	1.93 (0.81-4.62)	0.138	2.09 (0.87-5.05)	0.101
	54.49-615.31	35	75	4.51 (2.04-9.99)	0.0005***	5.83 (2.57-13.24)	p ≤ 0.001***
Urinary DMA (µg g^{-1} Cr) [c]	0.077-90.90	8	88	1.00 (ref)		1.00 (ref)	p ≤ 0.001***
	91.48-164.94	10	86	1.28 (0.48-3.39)	0.621	1.38 (0.52-3.70)	0.520
	165.42-302.10	19	77	2.71 (1.12-6.55)	0.026*	2.78 (1.14-6.77)	0.024*
	307.80-2353.5	29	81	3.94 (1.70-9.11)	0.001***	4.93 (2.08-11.64)	p ≤ 0.001***

CI, confidence interval, Cut off points were determined by quartiles of urinary arsenic metabolites of overall study participants.
p ≤ 0.05* , p ≤ 0.01**, p ≤ 0.001***
[a] ORs were adjusted by participant's occupation
[b] n=395, [c] n=385

Table 8.4. The logistic regression analysis of the ORs unadjusted and adjusted[a], for skin lesions risk in relation to urinary arsenic methylation indices

Urinary arsenic exposure measures (quartiles)		With skin lesions (n=66)	Without skin lesion (n=329)	Unadjusted OR (95% CI)	p-value	Adjusted OR[a] (95% CI)	p-value
iAs%	2.47-10.08	11	85	1.00 (ref)		1.00 (ref)	0.012*
	10.14-12.98	9	87	0.80 (0.32-2.03)	0.637	0.80 (0.31-2.06)	0.648
	12.99-17.0	19	77	1.91 (0.85-4.26)	0.116	1.79 (0.79-4.05)	0.160
	17.01-75.28	27	83	2.51 (1.17-5.39)	0.018*	2.65 (1.22-5.75)	0.014*
MMA%[b]	0.63-8.97	7	89	1.00 (ref)		1.00 (ref)	0.002**
	9.01-11.98	13	83	1.99 (0.76-5.23)	0.162	2.20 (0.83-5.84)	0.113
	11.98-15.90	16	80	2.54 (1.00-6.50)	0.051	2.72 (1.05-7.01)	0.039*
	15.92-42.62	30	80	4.77 (1.98-11.45)	0.0005***	5.06 (2.09-12.27)	p ≤ 0.001***
DMA% [b]	8.5-68.51	28	69	1.00 (ref)		1.00 (ref)	p ≤ 0.001***
	68.57-73.93	20	75	0.66 (0.34-1.27)	0.213	0.64 (0.33-1.26)	0.201
	73.98-79.02	9	87	0.26 (0.11-0.58)	0.001***	0.25 (0.11-0.56)	0.001**
	79.08,91.57	9	101	0.22 (0.10-0.49)	0.0005***	0.22 (0.10-0.50)	p ≤ 0.001***
PMI [b]	0.247-0.829	27	69	1.00 (ref)		1.00 (ref)	0.001***
	0.830-0.870	19	78	0.62 (0.32-1.22)	0.166	0.56 (0.28-1.12)	0.099
	0.870-0.899	9	87	0.26 (0.12-0.60)	0.001***	0.25 (0.11-0.58)	0.001***
	0.899-0.975	11	98	0.29 (0.13-0.62)	0.001***	0.28 (0.13-0.60)	0.001***
SMI [b]	0.293-0.814	30	67	1.00 (ref)		1.00 (ref)	p ≤ 0.001***
	0.814-0.856	15	80	0.42 (0.21-0.84)	0.015*	0.43 (0.21-0.88)	0.020*
	0.856-0.894	13	83	0.35 (0.17-0.72)	0.005**	0.34 (0.16-0.71)	0.004**
	0.895-0.976	8	102	0.18 (0.08-0.41)	0.0005***	0.17 (0.07-0.40)	p ≤ 0.001***

Cut off points of urinary were determined by quartiles of overall study participants;
p ≤ 0.05* , p ≤ 0.01**, p ≤ 0.001*** , [a] Adjusted by villager's occupation,
[b] n=395, [c] n=385

8.4 Discussion

This was the first cross sectional study to evaluate the dose–response relationship between arsenic exposure and skin lesions in rural Pakistan. Epidemiologic outcomes suggest that arsenic induced skin lesions although non-cancerous may convert to be cancerous with prolonged arsenic exposure (Haque et al., 2003; International Agency for Research on Cancer, 2004; National Research Council, 2001). Human methylation capacity plays an important role in determining arsenic induced disease susceptibility. It is therefore important to assess not only the arsenic methylation indices, but also the aggregated effect of these indices with population specific potential modifiers on arsenic-related disease risk. The population in the study villages was found mainly to be exposed to iAs (<1 to 3090 µg L^{-1}) from their household ground water sources. More than 89% of the household hand pumps exceeded the WHO provisional guideline value for arsenic in drinking water (10 µg L^{-1}), whilst 56% were also found to have iAs above Pakistan's water quality standard for arsenic (50 µg L^{-1}) (Rasheed et al., 2017a). The distribution of skin lesions indicated a lowest prevalence (0.7%) at 10-50 µg L^{-1}, 13.8% at 50-100 µg L^{-1} and 60% at >100 µg L^{-1}.Consequently, a higher prevalence of skin lesions was also found for those with higher daily arsenic intake. Past studies have reported the prevalence of skin lesions at iAs concentrations of <10 µg L^{-1} in China (Yang et al., 2017) and Bangladesh (Ahsan et al., 2006; Argos et al., 2011). Despite a very high arsenic exposure level for the current study population, the prevalence rate of skin lesions was found to be lower than the 22% reported in three villages of rural Bangladesh (Ahsan et al., 2000). Similarly, 41.8% was reported in Inner Mongolia for a population with an arsenic exposure level of 2.3-197.3 µg L^{-1} (Guo et al., 2006). Various demographic and life style factors affect arsenic methylation in arsenic-exposed populations such as age, sex, ethnicity, genetics, socioeconomic status, smoking, alcohol drinking, exposure route and duration, arsenic species, and nutritional inadequacy for essential vitamins, folate, N-acetylcysteine, glutathione, and zinc (Hsueh et al., 2016). The association between skin lesions risk and demographic characteristics was evaluated using univariate logistic regression. Age, sex, daily arsenic intake, village location, body weight and

occupation were revealed to be significant factors. A significantly higher prevalence of arsenic induced skin lesions in males (19.7%) than females (11.4%) suggests a higher susceptibility of males to develop skin lesions. These findings are consistent with other studies conducted in Bangladesh and elsewhere (Vahter et al., 1995; Argos et al., 2011; Rahman et al., 2006). The lower prevalence of skin lesions in female participants underscores the better methylation tendency of women than men, possibly linked with biological (hormones, physiology, genetics) and physical or social (sun exposure, water intake and smoking habits) differences between men and women.

Significantly increased skin lesions risk was found among older participants (>16 years) with an OR of 3.56 (95% CI: 1.25-10.152) compared to those ≤16 years. The probable reasons for higher age related susceptibility to arsenic-induced skin lesions include longer exposure duration, higher sun exposure due to the nature of occupation and daily water intake. Also, lower enzymatic and hormonal activity which are involved in arsenic detoxification, and old age related nutritional inadequacy and lower immunity may be the potential factors (Ahsan et al., 2006; Haque et al., 2003; Wei, 1998; Ahsan et al., 2007). Exposure duration to tAs from drinking water by participants with skin lesions varied between 10-20 years (tAs >100 µg L^{-1}), 14-20 years (As 50-100 µg L^{-1}) and 20 years for (As 10-50 µg L^{-1}) on the basis of consumption duration for household ground water. This suggests that the affected populations would be consuming untreated ground water for several years. Ground water tAs being the direct exposure variable seems to indicate the clear dose related trend for skin lesions risk above >10 µg L^{-1}. This is indicated by 20% increased risk of skin lesions for those exposed to 50–100 µg L^{-1} iAs compared to those with <10 µg L^{-1}, and this risk further increased more than 9.5-fold (OR 219, 29.14-1645.7) for the exposure >100 µg L^{-1} (Table 8.2).

The study showed that male, older, and/or heavier participants were more likely to be at risk of arsenic exposure (Table 8.2). An increased risk of skin lesions (OR 2.83, 95% CI: 1.48-5.39) was found among participants involved in labour intensive (farmers, wives of farmers and service providers like security guards, drivers etc.) occupations compared to the non-labour intensive (non-working house wives, students, tailors, teachers and un-employed) occupations (Table

8.2). Occupationally, the majority of the study participants were farmers (n=186) working outdoors and generally had sun exposure for 8-10 hours per day. The labour intensive occupations also included wives of farmers (n=56) contributing in the crop fields with their farmer husbands, possibly having higher sun exposure resulting in higher drinking water intake. The labour intensive occupations may also be associated with other risk enhancing factors such as low socio-economic status and poverty related malnutrition.

Simultaneous adjustment of significant confounding factors (Table 8.2) in multivariate regression analysis has showed an overall model significance for villager's occupation and thus adjustments were made for labour intensive and non-labour intensive occupation categories. This model adjustment was utilized to show that the association between skin lesions and urinary arsenic metabolites (tAs, iAs, MMA, DMA), methylation capacity (iAs%, MMA%, DMA%) and methylation efficiency (PMI and SMI) might be enhanced by intensive physical activities and higher sun exposure.

The influence of occupation is obvious from the decrease in adjusted ORs than unadjusted ORs for methylation capacity and efficiency indicators. Contrary to the studies by Haque et al. (2003) indicating ORs of 3.1 (51-99 μg L^{-1}), and 5.0 (>150 μg L^{-1}), and (Guo et al., 2006) showing ORs of 15.50 (51-99 μg L^{-1}), and 25.70 (>150 μg L^{-1}), this study showed much higher ORs of arsenical skin lesions for increasing arsenic exposure from household water sources. The impact of metabolically produced arsenic on the significantly increased skin lesions risk was obvious among the skin lesions subgroup in the 4th quartiles of urinary tAs, iAs, MMA, DMA, iAs% and MMA%, 2nd quartiles of DMA%, PMI and SMI.

A significantly increasing trend was found with increasing levels of urinary tAs (>247 μg g^{-1}) indicated by a 2.4-fold increased odds of skin lesions (Table 8.3). Compared to this, Argos et al. (2011) reported 2.4-fold increased odds of skin lesions at a comparatively higher level of urinary tAs (i.e. >393 μg g^{-1}). Intermediary by-products of iAs such as MMA and DMA are methylated via similar metabolic pathways, however MMA is considered more toxic than iAs and DMA (Chen et al. (2013). The trivalent forms of MMA produced in this process were considered to be more toxic than pentavalent MMA (Hirano et al., 2003; Petrick

et al., 2001). The limited evidence on the health risk potential of ingested arsenic compared to metabolically produced MMA or DMA has given impetus to assess the relationship between arsenic related health effects and methylation capacity. Following this, the study results showed the association of daily arsenic intake with skin lesions incidence in a dose-dependent manner for absolute concentrations of urinary arsenic metabolites (Table 8.3). Increasing ORs from lower to upper quartiles of urinary arsenic metabolites demonstrated that the magnitude of exposure is directly related to the presence of skin lesions. Sub-groups with skin lesions indicated significantly higher mean values of urinary iAs%, MMA%, lower DMA%, PMI and SMI compared to those without skin lesions (Table 8.1). These findings are also in close agreement with the studies by Steinmaus et al. (2006) and Kile et al. (2011), revealing higher levels of urinary MMA% related with the higher risk of lung cancer and skin lesions respectively.

Arsenic methylation mechanisms are still controversial, however the ORs for arsenic induced diseases have been found higher in those with higher MMA% (Chen et al., 2013; Zhang et al., 2014; Li et al., 2015). Of all the methylation indices determined in this study, MMA% in upper quartiles (OR 5.06, 95% CI: 2.09-12.27) indicated the highest skin lesions risk compared to its corresponding reference (OR 1.00). Comparing the current study findings with earlier studies, MMA% is suggested to be an underlying reason of higher dermatoxicity and also a potential biomarker for preliminary screening of individuals suspected to be at an arsenic induced health risk.

The significantly decreased risk of skin lesions in the fourth quartiles of DMA% (OR 0.22, 95% CI: 0.10-0.50) and SMI (OR 0.17, 95% CI: 0.07-0.40) was also in agreement with earlier studies on arsenic induced development delays (Hsieh et al., 2014) and skin lesions (Li et al., 2011)**.** The higher iAs%, MMA% and lower DMA% among the participants with skin lesions depicted inefficient methylation capacity compared to those without skin lesions. This association between inadequate methylation capacity and arsenic induced health effects was found to be consistent with studies on arsenic induced cardiovascular diseases (Chen et al., 2013; Li et al., 2015) and bladder cancer (Chen et al., 2003).

Participants with oral arsenic exposure >50 µg L^{-1} and also having skin lesions showed significant increased (p=0.004) urinary MMA concentration compared to those exposed to tAs through drinking water but without skin lesions. The study participants identified with skin lesions belonged to 47 households. Out of these 47, 20 houses comprising 53 study participants revealed 28 persons with skin lesions, while 25 persons from the same houses showed no skin problems, despite being exposed to the same level of arsenic from their household water sources (Figure 8.3).

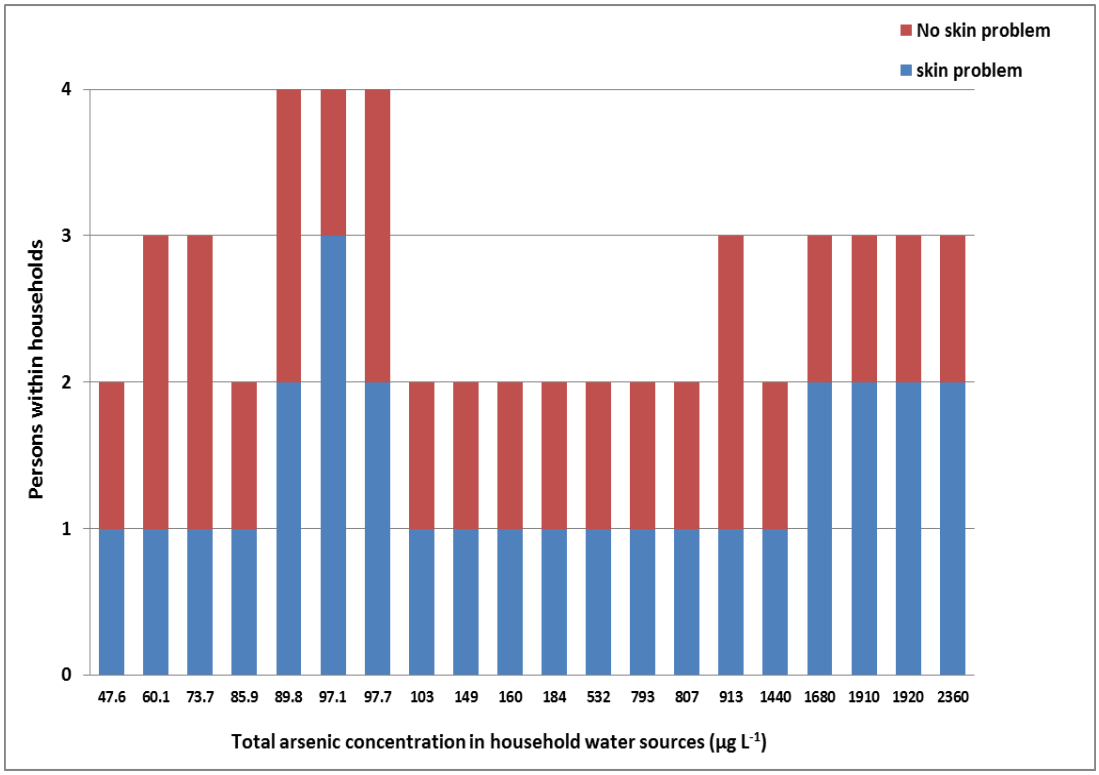

Figure-8.3: Households showing tAs concentration in ground water sources and inter-individual variability for arsenic induced skin lesions

Persons within the same house with higher arsenic concentration but with no skin lesions were found to be younger in age than their family members having skin lesions. The fact that some study participants did not develop skin lesions despite similar exposure to arsenic as those who did suggests the possible influence of inter-individual variability and various demographic, biological, genetic and nutritional factors on methylation efficiency. Valenzuela et al. (2009) found that genetic polymorphisms for arsenic (+3 oxidation state) methyltransferase (*AS3MT*) influence the susceptibility of humans to arsenical skin lesions and

these people might be at higher risk for other arsenic induced adverse health effects. Deficiency of nutrients such as proteins, folate, vitamin B_{12} and vitamin B_6 have been emphasized to interfere in arsenic metabolism and toxicity resulting in increased susceptibility to arsenic induced disease e.g. age-adjusted prevalence keratosis (Zablotska et al., 2008). This is indicated by positive correlation between urinary DMA and plasma folate in Bangladesh (Gamble et al., 2005) and negative correlation between the prevalence of arsenic-induced skin lesions and proteins intake (Mitra et al., 2004). Nutritional inadequacy may also be the reason for age related susceptibility to skin lesions, especially in case of older participants. The individuals with or without skin lesions might have suffered from other arsenic related health hazards which need to be further investigated.

The study findings may prove useful in understanding arsenic induced susceptibility to skin lesions, for early detection of skin lesions in communities residing in arsenic-affected regions, and may also be helpful for policy and decision makers. In addition to speciation for MMA, future studies should also evaluate the impact of association between arsenic methylation capacity and other modifiable risk factors on the variations in arsenic induced health hazards.

8.5 Conclusions

The occupation adjusted odd ratios suggested a significant dose response relationship between various exposure levels measured, using either water or urinary total arsenic, and the risk of skin lesions. The study supports the findings of other cross sectional studies demonstrating the inefficient methylation capacity in association with higher iAs% and MMA%, lower DMA%, PMI and SMI among individuals affected with arsenic induced diseases. The significantly increased risk of MMA% in older individuals with skin lesions indicates the metabolic barriers to converting MMA to DMA, also underscoring the probability of other arsenic induced health hazards among the exposed population. Even though skin lesions occur at exposure to 10-50 µg L^{-1} arsenic, countries including Pakistan currently follow a drinking water standard for arsenic of 50 µg L^{-1}. This may place many people at risk of developing arsenic induced adverse health effects with

persistent exposure. Our findings support an association between skin lesions and a higher intake of arsenic concentrations beyond the WHO provisional guideline value for arsenic in drinking water (10 µg L^{-1}).

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Chapter 9: Integrated health risk assessment for arsenic: multiple exposure sources and arsenic species

Rasheed H; Kay P**;** Slack R; Gong YY. Integrated health risk assessment for arsenic: multiple exposure sources and arsenic species. *(In Review in Environmental Health Perspectives)*

Abstract

Dietary exposure of a previously unstudied rural population to arsenic was assessed using an integrated risk assessment approach based on arsenic speciation, dietary consumption, socio-demographic data and specific toxicological reference values or general thresholds of toxicological concern. Probabilistic modelling resulted in a cumulative skin cancer risk of 97 in 10,000 persons, and mean hazard quotient of 53.4±95.8 due to inorganic arsenic (iAs) exposure: this risk was highest for children and women. Species-specific hazard quotient and cumulative cancer risk for arsenate (AsV) and arsenite (AsIII) were above the USEPA risk limits of 1.00 and $1x10^{-4}$ respectively, whilst dimethylarsinic acid (DMA) of dietary origin was not found to pose a risk. The excess lifetime risk of bladder and lung cancers was determined using this study parameters along with mortality:incidence ratios for Pakistan and USEPA dose-specific relative risk estimates for Blackfoot-disease endemic area of southwest Taiwan. Like skin cancer, females were at higher risk of developing bladder and lung cancer indicated by lifetime excess cancer risks of 51 and 7 compared to 46 and 4 for males in a population of 10,000 respectively. The study has also identified that a 67-year lifetime skin cancer risk for drinking water at the public health goal of 1 excess case of cancer per ten thousand people exposed was 2.50 µg iAs L^{-1}. Owing to risk assessment limitations, further research is needed to define the toxicological thresholds of arsenic species.

9.1 Introduction

Humans are exposed to arsenic (As) primarily via water and most frequently consumed food. Toxicity of inorganic (arsenate (AsV), arsenite (AsIII)) and

organic (monomethylarsonous acid (MMAIII), monomethylarsonic acid (MMAV), dimethylarsinic acid (DMAV) and dimethylarsinous acid (DMAIII)) arsenic i.e. varies significantly (Agency for Toxic Substances and Disease Registry, 2007). In the inorganic form, rapid cellular uptake of AsIII results in higher toxicity than AsV as reported by Agency for Toxic Substances and Disease Registry (2007) and this led to hypothesise that AsIII even at low concentrations would result in higher health risk. Inorganic arsenic (iAs) is reduced/methylated to MMA, which is further methylated to DMA via the highly reactive and genotoxic intermediate trivalent forms, MMAIII and DMAIII (Aposhian and Aposhian, 2006; Orloff et al., 2009; Wang et al., 2015). The formation of MMAIII may account for the toxicity and carcinogenicity of iAs resulting in cancer and non-cancer health effects. Based on the evidence of carcinogenicity in humans and animals, the International Agency for Research on Cancer (IARC) categorized arsenic and iAs compounds as 'carcinogenic to humans' (Group 1) and later classified DMA and MMA as 'possibly carcinogenic to humans' (Group 2B) (International Agency for Research on Cancer, 2012a).

A risk assessment that fails to consider arsenic species from food and assuming total arsenic (tAs) as being present solely as iAs would lead to an overestimated health risk (European Food Safety Agency, 2009). Most risk assessment studies have been conducted on exposure from water, while risks from AsIII, AsV, DMA and MMA in food are less understood. Though, rice being global staple have been well studied and showed to contain variable levels of arsenic species (Williams et al., 2005), the risk of arsenic species in wheat despite its higher global consumption than rice is less studied considering less arsenic accumulation in wheat than rice (Williams et al., 2007; Food and Agriculture Organization, 2017).

Considering such unmet need, this study aims to assess the influence of different exposure sources (water and food staples, rice and wheat) and the different arsenic species (iAs, AsIII, AsV, DMA) on health risk (skin, lung and bladder) using data from a rural population in Pakistan. More specifically, this study seeks to: 1) characterise the species-specific cumulative cancer and non-cancer risks due to combined exposure from water and dietary staples; 2) quantify the skin,

bladder and lung cancer risk due to iAs exposure; 3) determine the level of arsenic concentrations in relation to the acceptable risk level.

9.2 Methodology

9.2.1 Sampling design and study area characteristics

Six study villages. (Badarpur, Basti Balochan, Chak-46/12-L, Chak-48/12-I, Chak 49/12-I and Kotla Arab) located within four districts of Pakistan (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan) were previously found to have groundwater arsenic levels in excess of 10 µg L^{-1}. These villages consisted of 1776 households, with a population of 15647 (51% males; 49% females) and an average of 7 family members per house (Pakistan Bureau of Statistics, 1998). A description of the sample population (398 non-smoking volunteers representing 223 households from across the six villages) is provided in previous studies (Rasheed et al., 2017e; Rasheed et al., 2017d).

Secondary analyses of our previously published population-specific dietary consumption data (Rasheed et al., 2017e); laboratory results of tAs and arsenic species in drinking/cooking water (Rasheed et al., 2017d), rice and wheat (Rasheed et al., 2017a), hair, toenail and urine (Rasheed et al., 2017b) as well as examination of arsenical skin lesions in study participants (Rasheed et al., 2017c) was performed to conduct an integrated risk assessment. Since AsIII and V were analysed as total iAs, thus AsIII data were derived from analysed tAs of wheat and raw rice, using the assumption that %AsIII reported in rice is 60–90% (mean 75%) of tAs (Halder et al., 2014; Ma et al., 2016) and 38-71% (mean 55%) of AsIII in wheat (Cubadda et al., 2010). The derived AsIII and AsV from tAs of wheat and rice were included with the laboratory analysed tAs, iAs, DMA and MMA for an integrated health risk assessment of study participants up to age of 67 years (n=386).

9.2.2 Assessment of daily dose

The average daily dose (Equation 9.1) and the life-time average daily dose (Equation 9.2) was calculated for AsIII, AsV, iAs and DMA in water, wheat and

raw rice and for every study participant assuming 100% bioavailability for AsIII, AsV, iAs (United States Environmental Protection Agency, 2011; Laparra et al., 2005) and also for DMA based on its demethylation to iAs.

$$ADD_{x,i} = \frac{C_{x,i} \times IR_i \times EF_i \times ED}{AT \times BW} \quad (Eq.9.1)$$

$$LADD_{x,i} = \frac{C_{x,i} \times IR_i \times EF_i \times ED}{AT_e \times BW} \quad (Eq.9.2)$$

ADD	Average daily dose (mg kg^{-1} day)
LADD	Lifetime average daily dose (mg kg^{-1} day)
x	iAs or each of the arsenic species (AsIII, AsV, DMA)
i	Media: water, rice or wheat
C	Arsenic concentration: water (µg L^{-1}), rice/wheat (µg g^{-1}) (for unit consistency multiplied by 0.001 to get water as (mg L^{-1}) and rice/wheat as (mg kg^{-1})
IR_x	Ingestion rate: water (L day^{-1}), food (g day^{-1}) (for units consistency multiplied by 0.001 to get food as (kg day^{-1})
EF	Exposure frequency (days $year^{-1}$) i.e. 365
ED	Exposure duration (years)
AT	Averaging time is the period of time over which the exposure is relevant for health risk characterization (days/year).
ATe	Average life expectancy (days) = (365 days/year * 67 years)
BW	body weight (kg)

Total dietary exposure from each of iAs, AsIII, AsV, and DMA for each person was calculated using Equation 9.3 (ADD) and Equation 9.4 (LADD).

$$ADD_{total(x)} = ADD_{water(x)} + ADD_{rice(x)} + ADD_{wheat(x)} \quad (Eq.9.3)$$

$$LADD_{total(x)} = LADD_{water(x)} + LADD_{rice(x)} + ADD_{wheat(x)} \quad (Eq.9.4)$$

9.2.3 Assessment of health risk

Non-cancer risk is calculated for each study participant by comparing the individual exposure (ADD) to the toxicity value as a reference daily dose (RfD) via a ratio known as the "hazard quotient" (HQ). HQ is quantified for iAs, AsIII,

AsV, and DMA for each study participant using Equation (9.5) (U.S. Environmental Protection Agency, 1992).

$$HQ_x = \frac{ADD_{total\,(x)}}{RfD_x} \qquad (Eq.9.5)$$

Cumulative cancer risk (CR) was quantified as a probability of developing cancer by using the exposure and cancer slope factor for skin cancer in Equation (9.6): (U.S. Environmental Protection Agency, 1992).

$$CR_x = LADD_{total\,(x)} \times CSF_x \times ADAF \qquad (Eq.9.6)$$

CSF_x Cancer slope factor for species x

ADAF Age dependent adjustment factor

RfD and CSF are available only for iAs and have not been established for trivalent or pentavalent arsenic species (United States Environmental Protection Agency, 2011). A review of the literature allows toxicity thresholds to be derived: Table 9.1 lists RfDs (AsIII, AsV) and CSFs (AsIII, AsV and DMA) based on relative toxicity.

Table-9.1: Estimated RfDs (mg kg^{-1}-day) and CSFs(mg kg^{-1} $day)^{-1}$ for arsenic species used for cancer and non-cancer effects

Species	Derivation Criteria	RfD	CS factors	Source
iAs	Reflecting the dose-response relationship for skin related non-cancer and cancer effects	0.0003* (based on skin lesions/ Hyperpigmentation, keratosis and possible vascular complications)	1.5* (based on non-melanoma skin cancer)	US Environmental Protection Agency (1998)
AsV	Higher percentage of iAs is primarily reported as AsV in water hence the RfD and CSF of iAs applied for AsV.	0.0003*	1.5*	Markley and Herbert (2009)
AsIII	1.5 orders of magnitude higher toxicity of AsIII than AsV	0.000006*	75*	Markley and Herbert (2009)
	2-10 times higher toxicity of AsIII than As(V)	0.00003-0.00015	3-15	Goyer (2001)
	60 times higher toxicity of AsIII than As(V)	0.000005	90	Ratnaike (2003)
DMA	Two orders of magnitude less toxic than As(V)	0.03	0.015*	Markley and Herbert (2009) (estimated)

Species	Derivation Criteria	RfD	CS factors	Source
	$BMDL_{10}$ of 1.80 mg DMA kg^{-1} day^{-1} was divided by an uncertainty factor of 100**	0.02*		(Agency for Toxic Substances and Disease Registry, 2007)
	Based on the study by Arnold et al. (2003) indicating regenerative proliferation of the bladder epithelial from tissue in rats	0.014		US Environmental Protection Agency (2006)
MMA	The $BMDL_{10}$ of 1.09 mg MMA kg^{-1} day^{-1} was divided by an uncertainty factor of 100*	0.01		(Agency for Toxic Substances and Disease Registry, 2007)
	Based on the study by Arnold et al. (2003) indicating decreased body weights diarrhoea, body weight gains, food consumption, histopathology of gastrointestinal tract and thyroid in rats	0.03		US Environmental Protection Agency (2006)

*used in risk quantification in this study
**10 to account for extrapolation from animals to humans and 10 for human variability

In present study MMAIII and DMAIII could not be speciated due to challenges involved in controlling the rapid oxidation of MMAIII to MMAV, whereas MMA concentrations were not included in risk analysis due to levels detected in traces or below detection limits in water and food. Using a probabilistic approach (population risk assessment), the cumulative lifetime skin cancer risk resulting from combined exposure to iAs in water, rice and wheat was determined along with the non-cancer dermal effect. According to International Agency for Research on Cancer (2004a), the earliest symptoms of As exposure appear in skin, whereas AsIII has also been indicated as one of the co-carcinogenic agents in arsenic induced skin cancer in mouse (Cantor, 1997), thus probable skin related cancer and non-cancer risk for AsIII, AsV and DMA were also assessed using available toxicological information (Table 9.1). For non-cancer effects, HQ>1 suggests that there may be health concern (U.S. Environmental Protection Agency, 2016), whilst the USEPA acceptable cancer risk (CR) range is 10^{-4} to 10^{-6} which is dependent on the size of the target population (U.S. Environmental Protection Agency, 2017). Since the study area has a total population of 15646, an acceptable cancer risk of 1.0×10^{-4} (one case per 10,000 population) was

considered. In addition to population based health risk, the individual risk of study participants was also calculated as point estimates using equations (9.1 to 9.6) and were validated with previously assessed biomonitoring results of same study participants (Rasheed et al., 2017b) and prevalence of arsenical skin lesions (Rasheed et al., 2017c).

9.2.3.1 Defining the probability distributions for input variables

As concentrations, daily dietary consumption rates, body weight and age were described as probability density functions in @RISK (Version 7.5, Palisade Corp. USA) to identify the most appropriate probabilistic density functions based on Akaike information criterion (AIC), whilst other variables were kept as constant (Table 9.1).

Table-9.2: The input parameters used in probabilistic risk estimation

Input variable		n	Descriptive statistics	Probabilistic estimates*	Data source
Concentrations in water ($\mu g\ L^{-1}$)	iAs	228	Min-max: 0.48-3090	Inverse Gaussian Distribution	Rasheed et al. (2017b)
	AsIII		Min-max: 0.37-100	Pareto Distribution	
	AsV		Min-max: 0.11-3430	Inverse Gaussian Distribution	
	MMA		0.7-0.20	distribution not defined	
	DMA		0.14 -1.80	distribution not defined	
Concentrations in raw rice ($\mu g\ kg^{-1}$)	iAs	105	Min-max:63-200	Pareto Distribution	Rasheed et al. (2017a)
	AsIII**		Min-max:40-198	Derived from iAs***	
	AsV		Min-max:2-23	iAs - AsIII	
	MMA	10	0.25	distribution not defined	
	DMA		Min-max:0.25-23	ExtvalueMin Distribution	
Concentrations in cooked rice ($\mu g\ kg^{-1}$)	iAs	24	Min-max:18-300	Pareto Distribution	Rasheed et al. (2017a)
Concentrations in wheat ($\mu g\ kg^{-1}$)	iAs	8 (composite samples based on 189 sub samples	Min-max:76-228	Pareto Distribution	Rasheed et al. (2017a)
	AsIII***		Min-max:34.9-164	Derived from iAs**	
	AsV		Min-max:41-63	iAs - AsIII	
	MMA		Mean:0.25	distribution not defined	
	DMA		Mean:.25	distribution not defined	
Estimated daily intake (IR)	Water ($L\ day^{-1}$)	5	Age 3-6 years: 1.9	Log logistic Distribution	Rasheed et al. (2017c)
		61	Age 6-16 Years:2.9		
		332	Adults >16: 3.6		
		398	Overall mean: 3.5		
		398	Min-max:1-7		
	Wheat ($g\ day^{-1}$)	4	Age 3-6 years: 149	Weibull Distribution	
		59	Age 6-16 Years: 227.		Rasheed et al. (2017c)
		331	Adults >16: 402		

Input variable		n	Descriptive statistics	Probabilistic estimates*	Data source
		394	Overall mean 372		
		394	Min-max: 85-1200		
	Raw Rice (g day^{-1})	4	Age 3-6 years: 27	Weibull Distribution	Rasheed et al. (2017c)
		34	Age 6-16 Years:79		
		130	Adults >16:154		
		168	Overall mean: 136		
		168	Min-max:24-350		
	Cooked rice (g day^{-1})	168	Min-max:76-765 (mean 469 ± 202)	Weibull Distribution	Rasheed et al. (2017c)
Body weight (kg)		5	Age 3-6 years: 12	Kumaraswamy Distribution	Rasheed et al. (2017c)
		61	Age 6-16 years: 26		
		320	Adults >16: 63		
		386	Min-max: 9-105		
Exposure duration (ED) (years)	years	5	Age 3-6 years: 6-Age (picked by Monte Carlo)	constant	Rasheed et al. (2017b)
		61	Age 6-16 Years: 16-Age (picked by Monte Carlo)		
		320	Adults >16 Year: 67- Age (picked by Monte Carlo)		
		386	Overall ED: 64 years		
Average Life expectancy	years		67 (for Pakistan)	constant	WHO (2015)
Age	years	386	Min-max:3-67	Kumaraswamy Distribution	Rasheed et al. (2017c)
Averaging Time (AT)	days/ year		365	constant	
Age dependent adjustment factor (ADAF)			For 0-2 years = 10	constant	USEPA (2011b)
			Age 2-16 years =3		
			Age 16-67 years = 1		
Reference dose (RfD) (mg kg^{-1} day)	iAs		0.0003 (based on skin lesions/ Hyperpigmentation, keratosis and possible vascular complications)	constant	USEPA (2011a)
	AsIII		0.000005 (estimated)		Markley and Herbert (2009) Ratnaike (2003a)
	AsV		0.0003 (estimated)		USEPA (2011a)
	MMA		0.01		ATSDR,2016
	DMA		0.02		ATSDR,2016
Oral slope factor for non-melanoma skin cancer (CSF) (mg kg^{-1}-day)$^{-1}$	iAs		1.5	constant	United States Environmental Protection Agency (2011)
	AsIII		75 (estimated)		Markley and Herbert (2009)
	AsV		1.5 (estimated)		
	DMA		0.015 (estimated)		

*Distributions truncated at maximum levels of each input variables to overestimation by extrapolation
**Raw rice AsIII derivation formula: (0.2555 iAs+47.91)iAs/100
***Wheat AsIII derivation formula: Wheat AsIII: (0.1707 iAs+ 33.073)iAs/100

Inter-dependency between two or more input variables was done by copula fitting (Appendices-9.6 and 9.7).

9.2.3.2 Probabilistic Risk Assessment (PRA)

The health risk was modelled using equations (9.1 to 9.6) in @RISK software (Version 7.5, Palisade Corp. USA). Running the model for 100,000 iterations, the life time cumulative risk was calculated based on an average life expectancy of 67 years. Risk plots were derived as @RISK output of Monte Carlo simulations indicating cumulative density functions (CDFs) of the mean risk estimates with 95% confidence interval.

9.2.3.3 Sensitivity analysis

Sensitivity analysis was conducted in @RISK to generate the Tornado plots to rank the importance of each input variable to the simulated 95% percentile cumulative risk estimates using regression coefficients. Mapped values were generated to quantify the change in estimate given a one standard deviation (1SD) change in each variable. The uncertainty analysis was carried out using 'Advanced Sensitivity Analysis' of @Risk using an uncertainty factor of 3 to iAs RfD (United States Environmental Protection Agency, 2011) and 100 for DMA (Agency for Toxic Substances and Disease Registry, 2007). These uncertainty factors were applied separately on base values of both RfDs and CSFs.

9.2.3.4 Risk assessment for bladder and lung cancer

The proposed CSF values for arsenic (as iAs) related bladder and lung cancer in male and female are yet not approved by USEPA. Therefore, arsenic dose-response coefficient (b) of US Environmental Protection Agency (2010) determined for the arsenic related bladder and lung cancer mortality data reported by Morales et al. (2000) for the southwest (SW) Taiwanese population was used in this study. This epidemiological data was based on 43 villages (42 exposed villages and the reference population) including well water As concentration (0-934 μg L^{-1}), age (for 5-year band of ages 20 to 84), water intake rate (2 L day^{-1} for female and 3.5 L day^{-1} for male), Taiwanese male and female body weight (50 kg), non-water dietary intake (10 μg day^{-1}), male and female lung and bladder cancer mortality, and at-risk population in southwest Taiwan. The

arsenic dose-response coefficient (b) of Taiwanese population (932.629 and 295.870 for female and male bladder cancer, 243.03 and 74.371 for female and male lung cancer respectively) along with available data on background cancer incidence, age specific mortality, and population at risk for Pakistan (Institute for Health Metrics and Evaluation, 2016; International Agency for Research on Cancer, 2012b), body weight and water intake of present study were integrated in the USEPA'S BEIR IV relative risk model (US Environmental Protection Agency, 2010). This integration using Solver® (Microsoft Excel plug-in) enabled to compute mortality to incidence ratio (MIR) and upper confidence limits (UCLs) of the dose-response coefficient (b) for bladder and lung cancer mortality. UCLs were used to derive lowest effective dose (LED_{01}) representing the lower limit of range with 95% confidence of being the effective dose for one percent lifetime incidence risk in the Pakistan's population. The cancer slope factor (CSF) was derived from the upper 95% confidence limit on the 1% cancer dose LED_{01} (Equation 9.7). Using CSF in Equation (9.8), incidence unit risks for lung and bladder cancer for males and female participants exposed to iAs from water and staple food in six study villages was estimated.

$$Oral\ CSF\ (mg/kg-day)^{-1} \quad = \quad 0.01/LED_{01} \qquad \textit{(Eq.9.7)}$$

$$Unit\ risk\ (\mu g\ L^{-1}) \quad = \quad CSF \text{ x } 0.001 \text{ x } IR_{water}/BW \qquad \textit{(Eq.9.8)}$$

The incidence unit risk was multiplied with iAs concentrations to estimate lifetime bladder and lung cancer incidence for this study participants of age >22 years (106 females and 175 males).

9.2.4 Public health goal for iAs in drinking water

The maximum contaminant level goal (MCLG) was defined as the level of a contaminant in drinking water below which there is no known or expected risk to health and for carcinogenic chemicals like arsenic, MCLG is set at zero (US Environmental Protection Agency, 2009). Similar to MCLG, the public health goal (PHG) is a risk management initiative aimed at restricting cancer cases to no more than 1 excess cancer in 10,000 based on daily water intake on 2 litres for 70 years (Hering, 1996), Considering the arsenic carcinogenicity and using population specific variables of this study, PHG for iAs in drinking water was calculated using following formula (Hering, 1996; Brown and Fan, 1994).

$$PHG = \left(\sum_{16}^{67} \frac{R \times BW \times F_{iAs}}{CSF \times IR_{water}}\right).66^{-1} \quad \textit{(Eq.9.10)}$$

Where R is 10^{-4} or 1 extra lifetime cancer case per ten thousand exposed individuals, F_{iAs} is relative source contribution to iAs exposure due to drinking water and is 0.9 or 90% in this study, IR is daily drinking water consumption of 2.0 L day^{-1} and BW is 61 Kg. The total number of data sets for study participants from >16 to 67 years old was 66 (with daily water intake of about 2 litres).

9.2.5 Statistical analysis

The arsenic concentrations below detection limit (BDL) were assigned a value at half the detection limit values to avoid overestimation. The results of health risks were analysed using Microsoft Excel and SPSS 24 (IBM, New York, NY, USA) for descriptive statistics.

9.3 Results

9.3.1 Distribution of input variables

The results of probability distributions given earlier in Table 9.2 indicated that the concentrations of iAs in wheat and rice, and AsIII in water were best characterised by Pareto distribution, whilst concentrations of iAs and AsV in water by Inverse Gaussian Distribution. DMA concentration in rice was fitted by Extreme minimum distribution. Wheat and rice intake were best fitted by Weibull distribution, water intake by log logistic distribution, body weight and age by Kumara Swamy distribution.

9.3.2 Risk of skin cancer and non-cancer dermal effects induced by iAs

Probabilistic estimates of combined total daily intake of iAs from water, wheat and raw rice resulted in a simulated cumulative HQ (non-cancer risk) of 53.4± 95.8 and skin cancer risk of 0.00969 (97 persons in 10,000) (Table 9.3) due to higher exposure from water followed by wheat and cooked rice as reflected by the

cumulative distribution function of 95th percentiles of the study participants (Figure 9.1).

Table-9.3: Probabilistic estimates of lifetime (cumulative) risk of skin cancer and non-cancer skin lesions (as hazard quotients, HQ) at 95% CI due to iAs dietary intake

Exposure sources and age groups	HQ (skin lesions as the point of departure)			Skin cancer risk of iAs exposure		
	mean	LB	UB	mean	LB	UB
Water	49.184	48.594	49.775	0.0089	0.0088	0.0091
Raw rice	0.726	0.723	0.729	0.0001	0.0001	0.0001
Cooked rice	2.176	2.163	2.189	0.0004	0.0004	0.0004
Wheat	3.507	3.495	3.519	0.0006	0.0006	0.0006
*Combined exposure	53.417	52.823	54.011	0.0097	0.0096	0.0098
3-6 years	205.697	194.934	216.460	0.0270	0.0267	0.0273
6-16 years	116.342	114.053	118.632	0.0165	0.0163	0.0168
>16 years	42.342	41.796	42.887	0.0085	0.0084	0.0086

*Combined exposure from water, raw rice and wheat

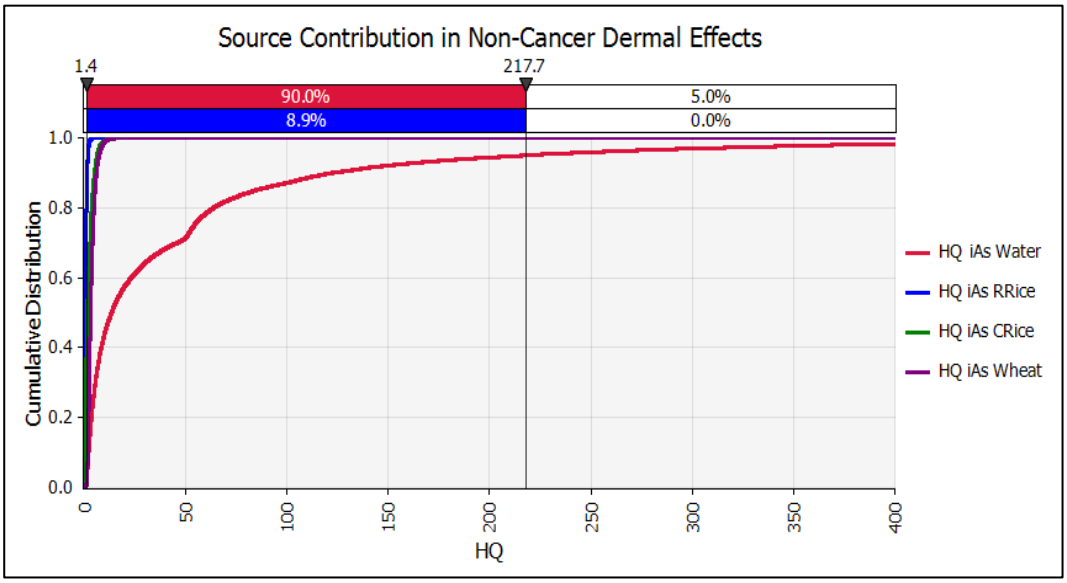

Figure-9.1: 95th percentile of cumulative probability distributions of iAs induced lifetime non-cancer risk as HQ (arsenical skin leisons): curves indicate iAs exposure from water, raw rice (RRice), cooked rice (CRice) and wheat. Red bar on top indicate 95th percentile of iAs induced non-cancer risk from water intake (HQ of 1.4-217.7), blue bar represents 95th percentile of iAs induced non-cancer risk from raw rice intake.

Exposure to iAs from an early age increases risk: for exposure at 3-6 years and 6-16 years results in a respective risk of 0.0270 and 0.0165 (270 and 165 children in a population of 10,000 respectively) compared to 0.0085 for adults (85 persons in 10,000). Nevertheless, all were above the USEPA acceptable cancer risk criteria of 1x10^{-4}. A similar pattern was observed for age-adjusted non-cancer risk (Table 9.3). The 5th percentiles of iAs related

skin cancer risk for highest contributing source (water) and lowest contributing source (raw rice) was 5% and 66.3%, whilst 90% and 33.7% of cancer risk were within 95th percentiles respectively as indicated in cumulative distribution function in risk plot (Figure 9.2).

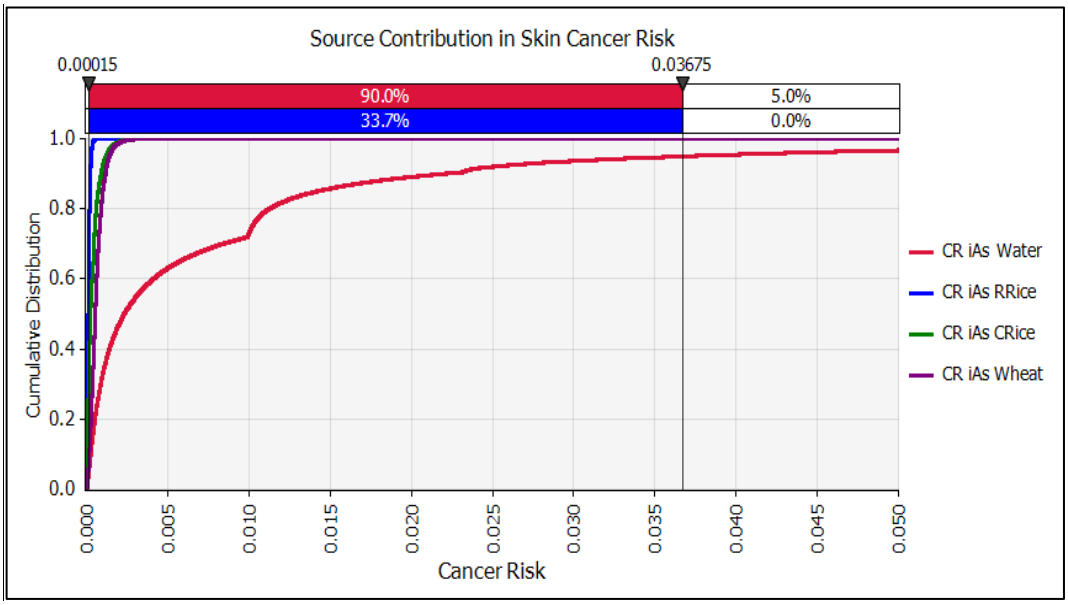

Figure-9.2: 95th percentile of cumulative probability distributions of iAs induced excess lifetime cancer risk (skin cancer): curves indicate iAs exposure from water, raw rice (RRice), cooked rice (CRice) and wheat. Red bar on top indicate 95% percentile of iAs induced cancer risk from water intake (0.00015-0.03675), blue bar represents 95th percentile of iAs induced cancer risk from raw rice intake.

Source wise the relative contribution of water (92%) and wheat (7%) intake in iAs induced cancer risk levels were higher than the raw rice (1%). Moreover, the geographical and gender differences were noted with females in two villages (Chak-48/12-I, Chak 49/12-l) exhibiting a higher cancer risk of 1182 and 401 persons in 10,000 respectively (Appendices 9.3 and 9.4).

9.3.3 Species specific cancer and non-cancer risk

Different arsenic species demonstrated different hazard quotients. Combined dietary exposure to AsIII resulted in the highest HQ of 192.51 followed by 53.25 (AsV) and (0.003) DMA (Figure 9.3). Exceedance of species specific HQ above the minimal limit of 1.00 was observed in 100% of the population for AsIII and AsV due to concurrent intake of groundwater, wheat and rice. Water was again shown to be the main exposure source, with raw rice the least.

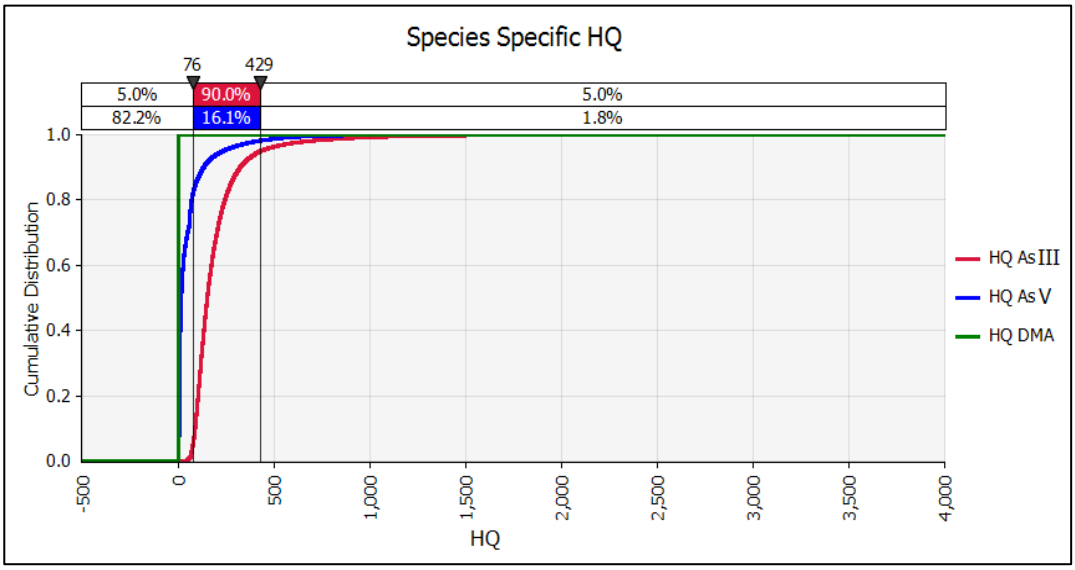

Figure-9.3: Species specific cumulative probability distributions of non-cancer risk (as HQ)

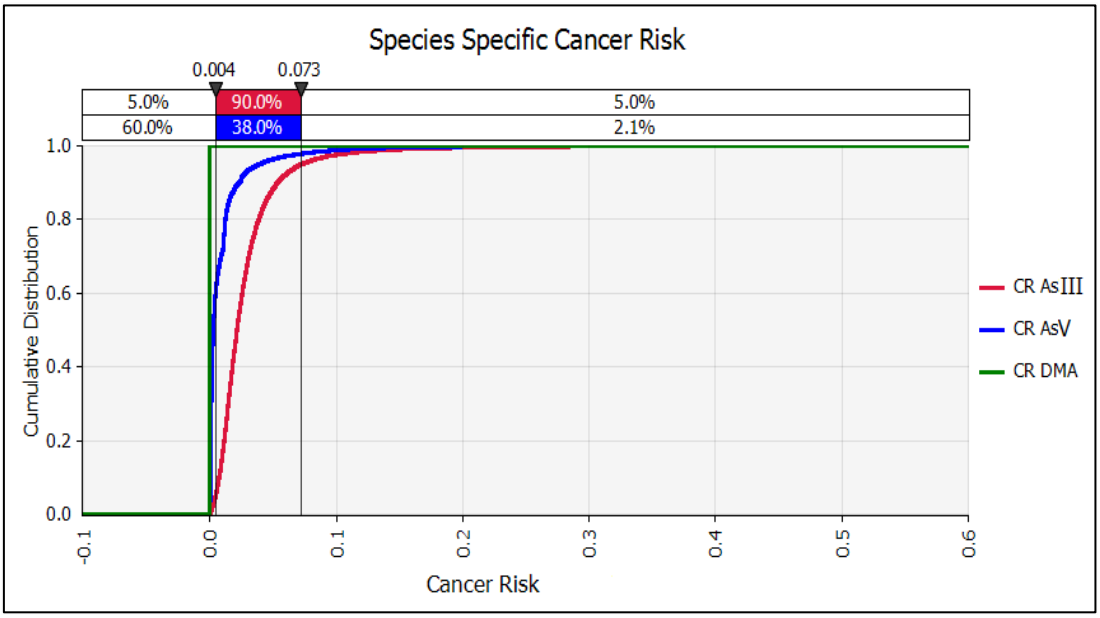

Figure-9.4: Species specific, cumulative probability distributions of lifetime excess cancer risk

Cumulative cancer risk followed the same pattern, with cancer risk highest for AsIII (284 per 10,000) followed by AsV (97 in 10,000) (Figure 9.4). The highest cumulative cancer risk was contributed by AsIII intake from wheat (53%) followed by AsV in water (97%). The mean cumulative cancer risk due to DMA was within the USEPA regulatory cancer risk limit ($1x10^{-4}$). Detail regarding species specific daily and lifetime exposure and contribution of sources in exposure and risk are given in supplementary information (Appendices 9.1 & 9.2).

9.3.4 Bladder and lung cancer risk (Internal cancer)

Table 9.4 shows the higher estimated oral CSF for female bladder cancer (0.54 per mg kg^{-1} day^{-1}) than males (0.36 per mg kg^{-1} day^{-1}), whilst the lung cancer oral CSFs for males and females were comparable. Drinking water unit cancer risks for lung and bladder cancer were higher for females than males, whilst LED_{01} was higher for males in both cases. Estimated drinking water concentrations associated with 10^{-4} lifetime incidence range from 4.43 µg L^{-1} (female bladder cancer) to 51.79 µg L^{-1} (male lung cancer).

The life time excess risk of bladder and lung cancer due to iAs exposure from water and food quantified for this study population is given as Table 9.5. The lifetime excess cancer risk for bladder cancer at iAs concentration <10 ug L^{-1} was 1 (in a population of 10,000) for males and females. Higher iAs concentrations correspond to higher estimated cancer risk per 10,000 individuals as shown in Table 9.5. The lifetime excess cancer risks of bladder and lung cancer were 46 and 4 for males, 51 and 7 for females, respectively. Overall lifetime excess cancer risks (per 10,000) from the two cancers were 54 per 10,000.

Table-9.4 Estimated risk metrics for lung and bladder cancers of this study population based on relative risk of Taiwanese population

Cancer type	iAs concentration in water (µg L^{-1}) at acceptable risk of 10^{-4}	Unit risk per ug L^{-1} drinking water	Oral CSF (mg kg^{-1}-day)$^{-1}$	1% Effective Dose Estimates LED_{01} (mg kg^{-1} - day)	Ratio of Taiwan's/Pakistan's total water intake	Mortality to incidence ratio (MIR)*
Bladder						
Male	4.90	2.01E-05	0.36	0.028	0.95	62%
Female	4.43	2.55E-05	0.54	0.018	0.63	81%
Lung						
Male	51.79	1.93E-06	0.03	0.294	0.95	88%
Female	32.26	3.10E-06	0.05	0.186	0.63	89%

*based on available incidence and mortality data for Pakistan (Institute for Health Metrics and Evaluation, 2016).

Table-9.5: Lifetime excess lung and bladder cancer risk estimates (per 10,000 populations) in study area

iAs concentration in water (µg L^{-1})	Bladder cancer				Lung cancer			
	Mean	LB	UB	% Population at risk	Mean	LB	UB	% Population at risk
Female (age 24 to 80 years)								
<10	1	1	1	0.01	0	0	0	0.00
10-50	6	5	7	0.06	1	1	1	0.01
50-100	17	16	18	0.17	2	2	2	0.02
100-200	30	27	32	0.30	4	4	4	0.04
>200	310	223	397	3.10	43	31	54	0.43
Overall	51	38	64	0.51	7	5	9	0.07
Male (age 23 to 80 years)								
<10	1	1	1	0.01	0	0	0	0.00
10-50	5	5	6	0.05	1	0	1	0.01
50-100	15	15	16	0.15	1	1	2	0.01
100-200	27	25	29	0.27	3	2	3	0.03
>200	280	201	359	2.80	26	19	34	0.26
Overall	46	34	58	0.46	4	3	5	0.04

Village wise the higher internal cancer excess risk was found in Badarpur (862 in 10,000), followed by village Chak 48 (129 in 10,000) (Appendix 9.5.).

9.3.5 Sensitivity analysis of Probabilistic risk estimates

Details on the sensitivity analyses are presented in the Appendices (9.8-9.13), briefly the sensitivity analysis for skin related probabilistic health risk due to iAs, AsIII, AsV and DMA showed that the most influential input variables for cancer risk model were AsIII (in water), age, AsV (in water) and wheat intake, whilst for HQ, BW, AsIII and AsV concentrations in water, and age were the most influential variables to affect the variance in non-cancer risk model prediction. For each SD increase in these variables, there was an increase in cancer risk (0.7-1.9%) and HQ (93-129.03), however age and body weight resulted in decreased risk by each SD implying decreased exposure with increasing age. Compared to these influential variables, the uncertainty factors of United States Environmental Protection Agency (2011) and Agency for Toxic Substances and Disease Registry (2007) integrated in this risk estimation showed higher influence on simulated cumulative risk.

9.3.6 Validation of health risk (point estimates) with bio-monitoring

The study participants with cumulative cancer risk above $1x10^{-4}$ also had higher concentrations of tAs, iAs (hair and toenail), urinary tAs, iAs, MMA and lower urinary DMA than those with lower cancer risk (Figure 9.5).

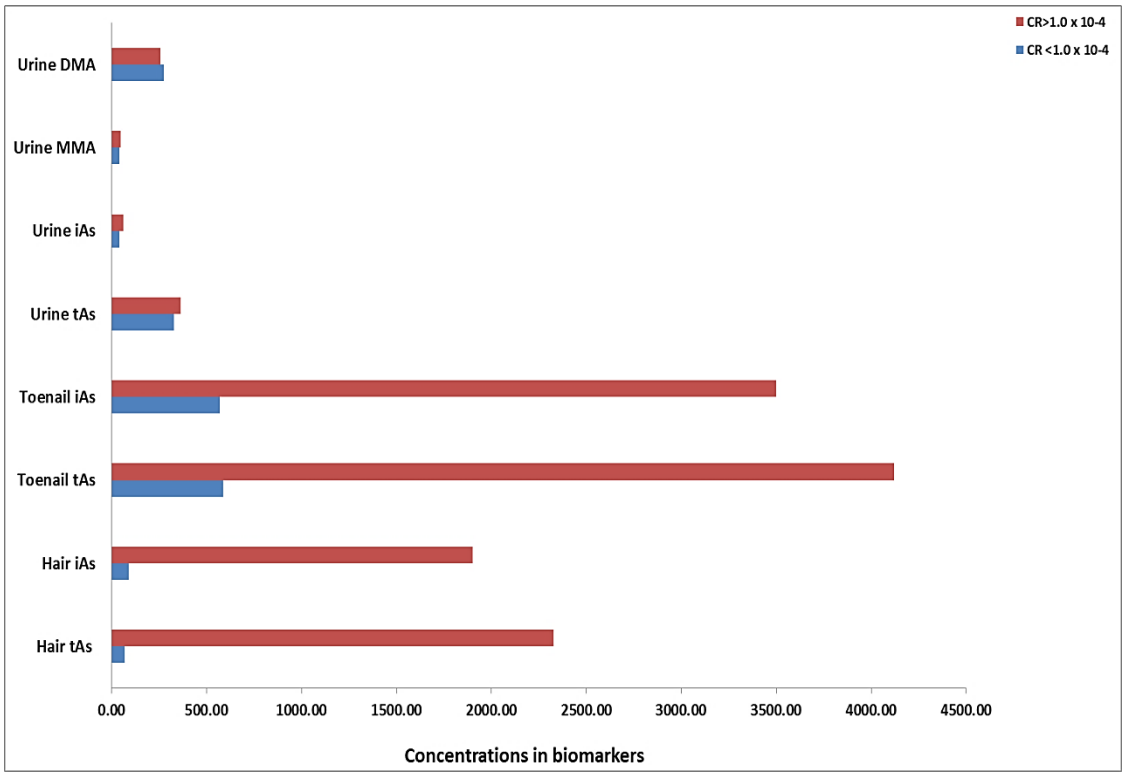

Figure-9.5: Concentration profile of As and species in urine, hair and toenail of study participants above and below the USEPA regulatory threshold target cancer risk level of 10^{-4}

Participants identified with arsenical skin lesions (Rasheed et al., 2017c) showed higher mean values of iAs related non-cancer (HQ of 255.804±265.890) and cumulative cancer risk (493 in 10,000 persons) than those without arsenical skin lesions (HQ of 91.661±182.611, cancer risk of 163 in 10,000).

9.3.7 Public health goal

The acceptable cancer risk of $1x10^{-4}$ with input variables of this study population was found at iAs concentration up to 2.77 µg L^{-1} in water, whilst the PHG calculated for iAs in drinking water was 2.50 µg L^{-1} based on the variables used in this study.

9.4 Discussion

The results suggest that AsIII intake via water and food, even at low concentrations, pose a three-fold increase in cancer risk compared to AsV due to combination of toxicity and exposure factors. The simulated risk estimates, along with a sensitivity analysis, indicate a reduction in cancer risk from AsIII > AsV > DMA; this is supported by earlier work (Petrick et al. 2000 and Abedin et al. 2002). The study also suggests that higher childhood exposure to AsIII, AsV or iAs may result in increased cancer risk in adulthood; this is supported by the literature (Nohara et al., 2017; Tokar et al., 2011). Since AsIII has been reported by Cantor (1997) as one of the causative agents in arsenic related skin carcinogenicity in mouse, the high doses also induced cancer transplacentally in the offspring in mouse tissues (Waalkes et al., 2003). Research into the toxicity of AsIII is ongoing but current understanding of toxicity, its mobility in water and higher levels in staple foods such as wheat and rice suggest that control measures are needed.

Contrary to earlier studies (Sharma et al., 2017; Sofuoglu et al., 2014) showing higher cancer and non-cancer risk due to iAs in rice, this study has indicated higher risk due to iAs in wheat as wheat is the main staple, higher consumption and higher iAs concentration caused higher exposure (21.5% from wheat vs 8.2% from rice) and cancer risk (7% from wheat vs. 1% from raw rice). Locally cultivated wheat in arsenic-affected areas is more likely to take up arsenic when irrigated with groundwater, resulting in bioaccumulation in wheat grains: as these areas do not grow rice, rice will be expected to have lower As levels (unless also grown in As-contaminated areas). In this study, cancer risk from consumption of raw rice (1 in 10,000) was not an established risk: risks of 4 to 7 persons (in 10,000) per 100 g per day rice consumption have been reported by Meharg et al. (2009) for Bangladesh, China, India, Italy and USA. Cooking rice in As-contaminated water increases As in the diet, with a cumulative cancer risk of 4 in 10,000 (Table 9.3) based on 469 ± 202 g day^{-1} of cooked rice.

The key determinants in the cancer risk model were AsIII (in water), age, AsV (water) and wheat intake rates, while body weight, AsIII (water), AsV (water) and age were factors for non-cancer risk. These findings differ from the results of

other risk assessment studies which found that rice consumption for a Turkish population (Sofuoglu et al., 2014), Spanish and US populations (Torres-Escribano et al., 2008; Yost et al., 2004), RfD and CSF of iAs for a Indian population (Pokkamthanam et al., 2011) were the main risk factors. This serves to demonstrate that different populations have different sources of exposure which also vary in extent. Uncertainty and within population variability will also be a factor but the spatially intensive approach used in this study can help to reduce population variability and uncertainties, yet the USEPA or ATSDR values we used or derived for As species such as RfD and CSF have their own uncertainty and highlight the need to address the gap of species specific toxicity threshold.

The lifetime excess cancer risk for bladder (1 per 10,000) and lung cancer (0 per 10000) at concentrations of iAs below 10 ug L^{-1} was found to be within the acceptable cancer risk limit of 1 x 10^{-4}. Compared to this, earlier studies using data from a Taiwanese population (Morales et al., 2000) have estimated lifetime excess bladder and lung cancer risk, as high as 23 (bladder) to 14 (lung) cases per 10,000 for males, 12 (bladder) to 18 (lung) cases per 10,000 for females (National Research Council, 2001). The US Environmental Protection Agency (2010) has shown risk of 32 (bladder) and 19 (lung) cases per 10,000 for males, 30 (bladder) and 48 (lung) per 10,000 for females at 10 ug L^{-1}.

Differences in cancer risk estimates might result from the use of different variables in the model parameters, for instance a high daily water intake was identified in this study villages (3.3 to 4.0 L day^{-1}) compared to the standardised variable often used in such models e.g. 2.0 L day^{-1} water intake recommended by United States Environmental Protection Agency (1997). In addition to water intake, the key influential factor in internal cancer risk was iAs concentration. Bladder cancer (0.27-0.3%, 29-32 persons) and lung cancer risk (0.03-0.04%, 3-4 persons) in a population of 10,000 exposed to 100–200 ug L^{-1} as reported in this study was similar to other studies which reported a slight risk increase as iAs exposure increased up to 150 µg L^{-1} (Morales et al., 2000; Lamm et al., 2013). A higher risk of bladder cancer at iAs concentrations in water above 200 µg L^{-1} was found in 3% of this study population. The studies by Lamm et al. (2014) and Lamm et al. (2015) showed that these internal cancer risk at lower iAs exposures

(<100-200 μg L^{-1}) continues to be debated, however the low dose (<200 ug L^{-1} iAs) risk estimates in this study population (0.01-0.3%) cannot be neglected due to continuous dietary exposure especially if coexposed to other risk factors including smoking. The literature showed higher synergistic effect of arsenic and smoking on bladder cancer at iAs concentrations below 200 ug L^{-1} (Kurttio et al., 1999), and lung cancer at below 11 ug L^{-1} (Ferreccio et al., 2013) and no effect at low doses (Meliker et al., 2010; Heck et al., 2009).

Though the current study participants were non-smokers, significantly more rural households in the study region were exposed to indoor tobacco smoke than urban households (45.2% versus 34.9%) as reported by Masud and Oyebode (2017) and may raise the arsenic related skin and internal cancer incidence among people co-exposed to dietary iAs and secondhand smoke as discovered by Ferreccio et al. (2013) and Melkonian et al. (2011) and hence requires further investigation on smoking and secondhand smokers in this study area.

A higher excess life time risk of bladder cancer in females (0.51%) than males (0.46%) was probably due to the difference of mortality to incidence ratio (MIR) in the study region. The higher MIR of female bladder cancer (81%) than males (62%) based on reported incidence and mortality rates (Institute for Health Metrics and Evaluation, 2016; International Agency for Research on Cancer, 2012b) resulted in higher slope factor and incidence unit risk (per ug L^{-1}) for females. The higher MIR of females than males for bladder cancer (81% vs 62%) used in this study were also consistent with the recent study by Wang et al. (2017) reporting higher MIR of females vs. males in Asian (50% vs 46%) and South East Asian (57% vs. 54%) regions of the world. Though, 94% of the females were reported not smoking in the study area, compared to 45% of smoking males (National Institute of Population Studies, 2013) and presumed as a cause of lower bladder cancer incidence rate of females than males, the higher mortality rates of females than males resulting in higher MIR of females (Table 9.4) may possibly be due to nutritional inadequacy, genetic polymorphisms, second-hand smoke and above all the limited access to advance health care facilities. Moreover, the lower ratio between female water intakes in the Taiwanese population and this study population (0.63) and a similar comparison of the two male populations

(0.95) also resulted in females to be at slightly higher cancer risk (Table 9.4). Species specific bladder and lung cancer could not be determined due to limited toxicological information, however fewer animal studies indicated implications of orally administered DMAV as urinary bladder carcinomas in rats (Arnold et al., 2006; Wei et al., 2002). DMAV when reduced to DMAIII resulted in cytotoxicity and regenerative cell proliferation and also concentrated and excreted in the urine (Cohen et al., 2006; Cohen et al., 2007). Though oral intake of DMA in this study is very low, the related effects of metabolically produced DMA cannot be ignored.

In humans, an inter-individual variation in arsenic metabolism may influence the person's susceptibility to cancer. Since cancer and non-cancer risk modelling estimates validated by bio-monitoring findings of same study participants demonstrated that the probability of fatal incidence of skin cancer was high for those identified with arsenical skin lesions and evidenced from their inadequate methylation capability and higher biological accumulation. Further investigation on histologically confirmed incident cancer case patients due to arsenic exposure may help to identify the possible impacts of species of dietary and metabolic origin and associated risk factors.

Finally, the high cancer risk estimates in this study demand the risk management initiatives such as compliance to the maximum contaminant level (MCL) of 10 μg L^{-1} iAs in water, establishing the PHG of iAs in drinking/cooking water as 2.50 μg L^{-1} determined in this study and defining the food safety limits based on dietary patterns.

9.5 Conclusions

In conclusion, we moved one step beyond the general risk modelling by including arsenic species, different exposure sources, and different health outcomes in a risk model. Combined exposure to iAs from water, rice and wheat resulted in comparatively higher skin cancer or non-cancer arsenical skin lesions in children and women than men, whereas this skin cancer risk was also comparable to previously identified skin lesion patients (Chapter 8). Based on input variables of this assessment including hypothetically derived cancer slope factors, species

specific cancer risks were higher for AsIII and vary mainly with age, AsIII concentrations and daily intake of wheat, while risk was lowest for DMA. AsIII exposure from water and food was the main concern and supports the study assumption that AsIII even at low concentrations is much more toxic than other arsenic species. The study highlights the need to establish toxicity-based slope factors for inorganic and organic arsenic species and for different cancer types. Since the debate over low-dose health risks from arsenic is inconclusive, this study integrates dose-specific relative risk estimates from southwest Taiwanese population with exposure characteristics for this study population, finding no risk of bladder and lung cancer at iAs ≤10 μg L^{-1}. Above 10 μg L^{-1} an increasing dose-response risk is found to be higher in females than males suggesting the integration of risk or confounding factors specific to both populations in future risk assessments. The health risk modelling estimates of this study were comparable to earlier bio-monitoring outcomes reflecting lower methylation tendency and higher arsenic accumulation in toenail and hair. To further refine this risk assessment process, future investigation needs to include the other dietary items, pathways (inhalation and dermal), MMAIII and DMAIII species and also integrate the human tissue and cellular concentrations of inorganic and organic arsenic species. The outcome of this study may allow refinement of risk assessment to enhance broad risk management strategies for the regulatory authorities.

9.6 References

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Chapter 10: Discussion

10.1 Research Synthesis

Arsenic is a known cause of skin, lung, bladder, liver, and kidney cancer, and also induces a wide array of other non-cancer effects to such an extent that no organ is untouched by its effects (Naujokas et al., 2013). Among the large variety of As species present in water, food, soil and biomarkers, AsIII and AsV have been considered the most toxic, whilst MMA and DMA have also been identified as cancer promoters and are mostly excreted as urinary metabolites (Batista et al., 2011; Signes-Pastor et al., 2016). Despite various *in vitro* and *in vivo* studies to understand metabolism of these species, there are still uncertainties regarding potential health risks, relative toxicity and toxicity thresholds of individual arsenic species in the human body. Since most risk assessment studies have assessed health risks on the basis of iAs in drinking water or rice, a limited understanding of the carcinogenic potential of individual arsenic species exists which provides a strong argument for the need to assess the species specific health risk.

This thesis assessed the cancer and non-cancer risk of arsenic species from the combined contribution of water and staple foods on a rural population from previously unstudied villages in the Punjab province of Pakistan. The main aims were to improve the risk assessment of human exposure to arsenic through better defining the exposure sources, pathways, intensity and health-related response indicators by adopting a spatially intensive approach. The study results presented in this thesis showed that age adjusted risk models revealed higher lifetime cumulative cancer and non-cancer risk for AsIII followed by AsV and DMA, supporting the hypothesis that dietary intake of trivalent and pentavalent arsenic species have a significant impact on health risks.

The population specific characteristics including water and food intake values showed twice the cancer risk than when computed with USEPA, WHO or reported intake values. This reflected the local situation, posing a higher risk of females developing skin, bladder and lung cancer due to iAs exposure from multiple sources. These findings agree with previous risk assessment studies based on exposure from drinking water. The study was novel due to age and gender

specific characterisation of most contributing exposure sources for arsenic species, their metabolism and health responses and ultimately integrating this comprehensive characterisation to conclude risk associated with individual arsenic species.

The deeper understanding of behaviour of species in exposure from water and high impact food, bioaccumulation, urinary excretion and methylation potential of every human of the study population permitted a realistic assessment of short term and long term health risks, providing evidence that these effects are also influenced by certain biological and behavioural modifiers such as age, gender, exposure level, occupation and exposure duration. Findings reported in this thesis also showed that arsenic intake from staple foods at low iAs concentration in drinking water varies with food consumption rates. Thus, any remedial measures to reduce arsenic exposure should consider persistent exposure from staple foods together with drinking/cooking water.

This study determined the age and gender specific daily direct and indirect water and food consumption rates and their impact on cancer and chronic health risks as well as urinary As metabolism. It has contributed with a rich data set to assess exposure and health risk of other chemical or biological contaminants, and developing the public health risk management plans. Based on the results, possible current exposure to arsenic via water used for drinking/cooking through use of shallow domestic hand pumps or dug wells, crop irrigation with arsenic contaminated tube well water or possible application of arsenical pesticides is a potential public health concern in the rural areas. This highlights the importance of effective exposure control initiatives by establishing public health goals for arsenic in public and private water sources, food safety limits, and consumption allowance of high impact food.

The present study concludes that the species specific health risk of arsenic is very complex, requiring highly controlled sample handling and analytical facilities, toxicity thresholds, reference doses or slope factors based on human studies or careful interpretation of animal toxicity models, bioavailability and uncertainty estimation. Since bioavailability and toxicological impacts of arsenic depend on its chemical forms, this study is anticipated to provide useful scientific information

and the possibility to compute health risk thresholds for the predominant arsenic species in dietary exposure sources based on available toxicity data. Further research is needed to help understanding of the distribution and inter-conversion of arsenic species of dietary and metabolic origin based on physiologically based pharmacokinetic modelling (PBPK). Moreover, integrating human tissue and cellular concentrations of arsenic species, histological responses and potential risk factors within a cohort might be beneficial to assist regulatory agencies to establish species specific toxicity or risk thresholds.

In the following paragraphs the author summarizes the main findings of the analytical chapters and how they contributed to achieve the objectives of this thesis.

10.1.1 Characterization of the potential sources of arsenic exposure

Using interview based 24 hours water diary method and food frequency questionnaire data was collected from residents of six rural settings on direct and indirect water and food consumption pattern and sociodemographic features (Chapter 4). The validity of modelling As related cancer risk was assessed using this consumption data above WHO provisional guideline value for arsenic in drinking water and earlier reported arsenic levels in wheat (Al-Othman et al., 2016) and rice (Rasheed et al., 2016) against standard or reported water and food intake rates for the USA, Europe and Asia.

This study data showed that age and gender specific water intake rates were 1-6 fold higher than the USEPA default (United States Environmental Protection Agency, 2011), World Health Organization (2005) recommended and the reported mean total daily water intake of Canada, USA, Europe, Latin American or lower than South Asian countries. This was attributed to different climatic and socio-economic conditions (occupation type), and different food and beverage intake patterns and preferences. These findings concur with studies in other geographical regions, for instance Drewnowski et al. (2013) reported a total water intake of 3.5 L day^{-1}, however their direct drinking water (37%) was similar to the indirect water intake of this study (24%).

Wheat was found as the main staple food with intake rate 2-10 fold higher than reported for USA, Europe and Asian sub-regions, whilst average daily rice intake

was found to be 3-4 fold lower than high rice consuming countries in South Asia. The validation demonstrated that using the default, standard or reported water intake values based on developed world populations or intake without including indirect water may underestimate the cancer risks for large numbers of people working in hot and humid environments as in this study area.

The lower rice in this study than higher rice intake countries (Bangladesh and India) allowed the preliminary evaluation of more reliable associations between rice and health risk. The use of available low range data of arsenic levels in wheat, despite the higher daily intake of wheat in this study, resulted in minimal cancer risk. The characterization of the potential sources of arsenic exposure based on population specific dietary choices and consumption frequency hold a key contribution in reducing the uncertainty inherent in the risk quantification process. This characterisation for a rural population was further used in various assessment scenarios presented in this thesis such as arsenic species exposure assessment (Chapters 4-6), biological monitoring (Chapters 7 & 8), modelling for As and its species specific non-cancer and cancer risk (Chapter 9). Exploring the association between diet and health risk by expanding the 24 hours dietary record to 7 days data collection is recommended as future work. Overall, the age, gender and occupation adjusted direct and indirect water and food intake data helped to bridge the dietary epidemiological research data gaps, understand the diet disease relationship in a regionally diverse setting affected by arsenic and identify the most exposed population sub-groups.

10.1.2 Relative contribution of arsenic species to human exposure and metabolism

This research study using an extensive chemical analysis has ascertained the type and concentration of arsenic species in water (Chapter 5) and food (Chapter 6) and how they are metabolized by the body by assessing biomarkers for recent and long term exposure (Chapter 7 and 8)**.**

The tAs concentration in domestic ground water sources was comparable to the high arsenic zones of the world having up to 5000 µg As L^{-1} in ground water even with some geological or hydrogeological variations as reported by Smedley (2008). AsV was found as the main species, whilst co-existence of AsIII with AsV

above 10 µg L^{-1} in village Chak-49 and the dominance of AsIII up to 100 µg L^{-1} was anticipated to be the result of variations in aquifer redox conditions. This variation was presumed to be associated with quaternary alluvial-deltaic sediments in the study region allowing aggregation of iron resulting in the onset of reducing conditions as explained by Smedley (2008). The higher concentration of AsIII and ratio of 3.3 between AsIII and AsV concentrations concurs with a study in the Blackfoot disease endemic area of Taiwan by Chen et al. (1995), indicating a ratio of 2.6 between AsIII and AsV. The shallow ground water sources (10 to 31 meters depth) in this study could be drilled further to remediate AsV but may not reduce the more toxic AsIII, as evidenced by the study of Erban et al. (2013) in the Mekong Delta in Vietnam due to pumping-induced clay compaction expelling arsenic to deep aquifers.

The raw rice consumed in the study villages was identified to be iAs type, similar to other Asian rice and contrary to US rice (DMA type), based on higher iAs concentration (>80%) than DMA and strong association between iAs and tAs in rice. Since iAs type was determined to be more toxic than DMA type rice, demethylation of DMA in rice crops as stated by Chavez-Capilla et al. (2016) may also result in higher iAs concentration in rice grains. However, uptake of As species in rice varies geographically depending on the crop variety, soil and irrigation water chemistry (Phan et al., 2014). The tAs concentration in locally grown wheat grains preferentially using ground water irrigation was higher than in raw rice cultivated beyond the current study districts. Though rice has a higher capacity to uptake As than wheat, arsenic rich irrigation water, soil or manures may be important sources for higher iAs concentrations in wheat (>99%) in this study, and can even go up to 740 µg kg^{-1} as determined by Norra et al. (2005). DMA and MMA in water and wheat were found in traces or were undetected.

The physical process such as milling may result in decreased tAs contents in both wheat and rice, whilst 7 times increased exposure of iAs from cooked rice than raw rice was attributed to arsenic uptake from high arsenic cooking water. Similarly, wheat flour kneading with high arsenic water not studied yet was also anticipated to further increase the iAs exposure than determined in this study.

Data from Chapter 4 on population specific consumption patterns were used to assess the relative contribution of As species in chronic exposure (Chapters 5 and 6). The estimated daily intake of iAs (mainly as AsV) from domestic ground water sources was comparatively higher than previously reported exposures in Pakistan and various As affected areas in Vietnam, Turkey, USA, India and Bangladesh. Though the relative contribution of water to estimated daily iAs intake was higher followed by wheat, the exceedance of most study participants beyond PTDI of 2.1 µg day^{-1} kg^{-1} body weight was higher due to water and cooked rice.

The combined average total daily intakes of tAs were consistent with the earlier reported level of 10 to 40 µg $kg^{-1}day^{-1}$ bw to cause cancer and non-cancer health effects (Lasky et al., 2004, Lubin et al., 2000; Kurttio et al., 1999; Hsueh et al., 1995). Exposure to DMA from raw or cooked rice was 8 times lower than respective iAs exposure, however considering DMA a possible carcinogen by International Agency for Research on Cancer (2012b), DMA cannot be ignored for long term daily rice consumption. At lower concentration levels of <10 µg iAs L^{-1} in water, a considerable exposure from rice and wheat have raised the question about prolonged low dose exposure. Since health implications from low dose iAs exposure solely from food is still debated, the available evidence associated the low dose As exposure with enhanced risks for diabetes, cardiovascular disease, immunological problems, and cancer (Schmidt, 2014). Children and females being more sensitive to toxic elements and identified at higher exposure levels were also expected to be vulnerable to low dose exposure.

The human metabolic process in this study caused 70% of the ingested tAs eliminated as urinary As metabolites, whilst the remaining 30% was assumed to be internally absorbed and/or excreted in faeces. The impact of As exposure on the internal dose of arsenic species under the influence of potential modifiers was observed (Chapter 7).

MMA has been reported as a highly reactive intermediate toxic metabolite produced during sequential methylation of iAs into DMA and has been associated with various cancer and non-cancer health effects. No oral exposure of MMA was

found, however the metabolically derived urinary MMA was of lower concentrations among female study participants than males and anticipated to be related to estrogen in women of childbearing age as reported by Lindberg et al. (2008). Metabolic DMA is believed to be readily eliminated in urine, accelerating the As detoxification from the human body. The higher level of DMA in urine and toenail of participants engaged in non-labour intensive occupations was surprising and presumed to be due to higher biotransformation of iAs into DMA, a part of which was excreted and a part accumulated in toenail and hair.

Furthermore, the impact of urinary arsenic metabolites and arsenic methylation potential on disease susceptibility, as such scientifically unstudied in this study area was also evaluated by examining the prevalence of arsenical skin lesions in the study villages.

The dose response effect for As induced skin lesions was clear at a level >10 µg As L^{-1} in water with an exposure duration of ≥10 years and with a daily As intake 10 times higher than those without skin lesions. This was also evidenced as higher levels of urinary iAs, MMA, MMA% and lower levels of DMA% and SMI revealing lower methylation capacity than those without skin lesions. These findings were in agreement with the studies by Steinmaus et al. (2006) and Kile et al. (2011), associating higher levels of urinary MMA% with higher lung cancer and skin lesions risk respectively. The influence of behavioural, biological or genetic effects on methylation capacity was obvious indicating inter-individual variability in skin lesions prevalence among members of the same families exposed to similar As levels. In this context, a further investigation revealed a strong significant association of tAs dose from water and food with urinary metabolite levels. Adjusting for gender, occupation and exposure durations suggested response modification by socio-demographic variables. The gender adjusted correlation between exposure from water and urinary arsenic metabolites was similar to the findings by Normandin et al. (2014). Arsenic speciation of hair and toenail have been inadequately conducted in the past considering these as metabolically inactive tissues. Since accumulation of As and its species in toenail partly depend on their concentration in blood, arsenic speciation of hair and toenail was performed to avoid misinterpretations of

biomonitoring data. The mean change in toenail tAs, iAs, MMA, DMA and hair tAs and iAs with one unit of change in the water and food tAs intakes have determined water to be a stronger predictor than food under the influence of gender, labour or non-labour occupations and exposure duration. The strong association of all toenail arsenic species was considered as indicative of critical health effects due to prolonged exposure.

Since association of tAs intake from water and food with As species in hair, toenail and urine has been assessed partially in the past, this study has resulted in better understanding of exposure–biomarker relationships and chemistry of arsenic toxicity, impacted by certain biological and behavioural modifiers. Further appraisals would be better to be focused on spatial variation of AsIII at various depths within an aquifer, bioaccessibility of arsenic species of wheat and the impact of other waterborne chemicals on arsenic metabolism.

10.1.3 Integrated health risk assessment approach

Considering the inadequate health risk assessment of integrated exposure of water and food and the influence of different arsenic species, iAs and species specific cumulative cancer and non-cancer risks were assessed based on available toxicological information (Chapter 9), average and life time daily dose of iAs, AsIII, AsV and DMA determined from estimated daily intake of water and food (chapters 5 and 6). The population based probabilistic model predicted iAs related cumulative skin cancer risks to affect 97 persons in 10,000 which was comparable to the prevailing skin lesions cases (Chapter 8). 5% excess risk was observed in affected and 2% in unaffected persons concluding that persons suffering with arsenical skin lesions are at higher risk of conversion of ongoing non-cancer effects into skin cancer, if the current level of exposure is not reduced. Species specific cancer risks were higher for AsIII contributed mainly by AsIII wheat (53%) followed by water (32%), whilst for cancer risk of AsV the main contributor was water (97%). The difference in toxicity and exposure levels suggested that AsIII intake even at lower concentrations poses the risk of developing cancer 3-folds higher than AsV. In addition to AsIII and AsV, concentrations in water are the most influential factors to increase the cumulative cancer risk by 1.9% and 1.7% respectively. Other determining factors sensitive

to risk estimation were age, BW and wheat intake and can contribute in model uncertainty, which was minimized by adopting a spatially intensive data collection approach. In addition to this population based probabilistic risk, non-cancer risk based on individual assessment as a ratio between daily intake of iAs and RfD (USEPA) or PTDI (WHO) or MRLs (ATSDR) for chronic and acute exposures (Chapters 5 and 6) showed children (≤16 years) as the most vulnerable group. This suggests increased risk potency due to a difference of body weight, exposure levels and metabolic rates than adults.

Furthermore, using dose specific relative risk estimates from the Blackfoot-disease endemic area of southwest Taiwan and mortality to incidence ratio (MIR) from Pakistan, life time bladder cancer risk was higher in females (0.51%) than males (0.46%) due to higher MIR of female bladder cancer (81%) than males (62%), and the lower ratio between female water intakes of Taiwanese and this study population. The higher female MIR due to bladder cancer was presumed to be related to nutritional inadequacy, genetic polymorphisms, secondhand smoke and limited access to advance health care facilities. Higher reported bladder cancer incidence in males by International Agency for Research on Cancer (2012a) and Institute for Health Metrics and Evaluation (2016) was anticipated due to persistent exposure to arsenic or other chemicals, higher smoking rates of males and inadequate methylation tendency. Contrary to this, Baris et al. (2016) have reported almost no gender difference in smoking rates among men (55.4%) and women (50.8%) and consequently in arsenic induced bladder cancer. Moreover, low dose internal cancer risk is still controversial. This study showed no risk at iAs concentration <10 ug L^{-1} and increased up to 0.3% for bladder and 0.04% for lung cancer risk at concentrations up to 200 ug L^{-1}, whereas above this the risk increased up to 3% for bladder and 0.07% for lung cancer in a dose response manner. Since this study population comprised only non-smokers, secondhand smoke and persistent low dose risk in the rural houses cannot be ignored as significant risk factors for synergistic effect.

Since the uncertainty of carcinogenicity of arsenic species from dietary and metabolic origin is yet not solved, the integration of biological (Chapter 7) and modelling estimates (Chapter 9) helped to quantify the lifetime cumulative risk

realistically. Persons with skin cancer risk above $1x10^{-4}$ were also found to have higher biomarker concentrations of As and its species, higher capacity to methylate arsenic to MMA and a lower capacity to methylate MMA to DMA. Further investigation of histologically confirmed incident cancer case patients may help to identify the influential risk factors for arsenic exposed population.

At 50 µg L^{-1} of iAs in water, an unacceptable cancer risk was found, whilst the acceptable cancer risk of $1x\ 10^{-4}$ with this population characteristics were indicated at iAs concentration up to 2.77 µg L^{-1} in water, which is also comparable to the public health goal of 2.50 µg iAs L^{-1} for iAs calculated in this study (Chapter 9). This may not be economically achievable, however the return in terms of reduced health incidence will be substantial. The preliminary advisory level of iAs in raw rice (200 µg kg^{-1}) as advised by Codex Alimentarius Commission (2014) was achievable in this study region with rice consumption of ≤200 g day^{-1} and compliance with ≤10 µg L^{-1} iAs in drinking water (Chapter 6).

10.2 Conclusions

In this thesis, I presented a comprehensive study of the source, exposure pathways and response elements of the human health risks of inorganic and organic arsenic species occurring in water and food due to geogenic origin. Cumulative cancer risk based on age and gender specific water and food consumption pattern of this study population against standard or reported water and food intake levels revealed substantially higher cancer risk due to water and wheat intake and lower cancer risk due to difference of rice intake.

Exposure assessment revealed higher intake of AsV from water followed by AsIII from wheat and rice, whilst DMA in rice was of lowest concern. Nevertheless, the predominance of AsIII in one village was indicative of a reducing environment in the ground water aquifer and higher toxicity. Arsenic and its species from staple food, alone and in combination with intake from direct and indirect water contributed significantly to exposure. In addition, arsenic intake from food offered particular significance where arsenic is relatively low in water (<10 µg L^{-1}) presenting wheat consumption as an alternative exposure pathway. In addition, evaluating an association of relative contribution of water and staple food with

human biomarkers provided a better knowledge of metabolism, urinary elimination and bioaccumulation of ingested arsenic species under the influence of certain biological and behavioural modifiers demonstrating toenail to be an effective biomarker of arsenic exposure of dietary or metabolic origin. Furthermore, the assessment of dose-response relationships between arsenical skin lesions and arsenic exposure offered a clearer understanding of the impact of inefficient arsenic methylation capacity on the increased risk of skin lesions under the influence of labour intensive occupations. Exposure from staple food and water used for drinking and all food preparations were found as the primary contributors of arsenic related skin cancer or non-cancer risk also evidenced with the inadequate urinary methylation capacity, higher arsenic species in toenail and hair as well as prevalence of skin lesion patients in high risk sub-groups. The appraisal of the potential risk of arsenic species offered important insights for identifying key species in producing carcinogenic and non-carcinogenic risk such as AsIII depending on age, concentration and daily intake of wheat. Moreover, the assessment of arsenic induced bladder and lung cancer among exposed populations outlined the higher sensitivity of females depending on their higher mortality to incidence ratio than males. By including both biomonitoring and risk modelling this study has contributed extensive data, providing an integrated approach for exposure, metabolism and risk assessment, especially for regulatory purposes and may instigate efforts to establish arsenic food safety standards. This integrated assessment provides a basis for conducting an integrated risk management based on water, food, agriculture and health. In addition, the understanding of exposure parameters for population subgroups offers the opportunity to propose minimum margins of safety for water and rice.

10.3 Recommendations for future research

a. This assessment has focused mainly on arsenic speciation in shallow ground water, further studies including a more substantial speciation of water and sediments samples from deep aquifers will be valuable for a better understanding of mobility and toxicity of arsenic species in variable geochemical and hydrogeological environments.

b. This study has focussed mainly on arsenic species assessment in wheat, rice, human urine and a limited number of hair and toenail samples. There is a need for further research to better characterize arsenic species exposure from other crops, livestock and poultry products. For implementing better agricultural management, studies on uptake of arsenic in wheat from soil, manures and pesticides as well as arsenic bioavailability in the human body after wheat ingestion are recommended.
c. Biomarkers for arsenic species were limited by the dearth of speciation analysis on trivalent methylated arsenic species in human biomarkers. Speciation analysis for urinary MMAIII and DMAIII from a controlled study of dietary intake over a period of weeks will be valuable for a better understanding of arsenic methylation and disease development mechanisms.
d. Studies on the association between arsenic exposure and skin lesions in relation to nutrients or energy intake and arsenic metabolism may clarify the inter-individual variability in arsenic induced health effects and methylation capability.
e. Further investigation to compare the exposure risk relationship with histologically confirmed incident cancer case patients within a population may help to identify the influential risk factors for arsenic exposed population. Likewise, these studies may produce useful toxicity data to be incorporated in cancer risk modelling.
f. Considering the scalability and sustainability of water treatment, techniques such as *In situ* treatment of arsenic contaminated groundwater could be explored to provide arsenic free water in the study area.

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Appendices-Chapter 3

Appendix 3.1 Ethical approval from University of Leeds

Hifza Johar Rasheed
School of Geography
University of Leeds
Leeds, LS2 9JT

ESSL, Environment and LUBS (AREA) Faculty Research Ethics Committee
University of Leeds

19 April 2018

Dear Hifza

Title of study: **Probabilistic Arsenic Exposure Assessment and Attributable Health Risks in Pakistan**

Ethics reference: **AREA 14-005 response 2**

I am pleased to inform you that the above research application has been reviewed by the ESSL, Environment and LUBS (AREA) Faculty Research Ethics Committee and following receipt of your response to the Committee's comments, I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	Version	Date
AREA 14-005 RESPONSE-3 final.docx	1	01/12/14
AREA 14-005 Information sheet, consents and questionnaires.doc	1	01/12/14
AREA 14-005 Hifza Rasheed Biological Specimen Reception Letter 6.2.14.pdf	1	01/12/14
AREA 14-005 FIELDWOR.DOC	1	01/12/14
AREA 14-005 Urine Importation Certification Letter 7.3.14.pdf	1	01/12/14
AREA 14-005 Ethical_Review_Form_HifzaJohar.doc	2	10/11/14
AREA 14-005 Information leaflet - main study.doc	2	10/11/14
AREA 14-005 DATA RECORDING FORMATS_Optional Study.doc	2	10/11/14
AREA 14-005 response.doc	1	10/11/14
AREA 14-005 Fieldwork_RA_form_Arsenic Work 2014 (Final).doc	1	11/08/14
AREA 14-005 FINAL FIELD FORMS Aug 2014.pdf	1	11/08/14
AREA 14-005 NBC Pakistan_ethical approval.jpg	1	11/08/14

Committee members made the following comments about your application:

"Arsenic occurs naturally in many wells and aquifers but at very high levels, may lead to health problems. To investigate levels of arsenic in your village, our research team will take samples of your food and water" Looks much better. The committee advises switching the location of the comma in the first sentence, as it currently might imply high levels in all wells, so: "Arsenic occurs naturally in many wells and aquifers, but at very high levels may lead to health problems." Likewise, you might add "potential" to the second sentence: "To investigate potential levels of arsenic in your village, our research team will take samples of your food and water", though researchers may feel this suggests an overly naïve take on the situation.

"Text has been updated accordingly but please advise if there is a standard approach for seeking consent for use of photographs of individuals." The University doesn't have advice on this, however the committee confirms that your approach would be the one that it recommends, ie. to avoid taking full face photographs where possible, or publishing them without blanking out irrelevant features if they are taken, and to explain the level of potential risk. Many thanks for this clear elaboration.

"The health system in rural areas is not well organized." Many thanks for the details on the field site situation. We are happy, in the absence of regular local medical relationships, for you to make contact with the families directly, trusting the researchers that this will be done with appropriate sensitivity, provided the disclosure is appropriately worded, e.g. "What you have is certainly similar to conditions caused by arsenic, but we're not doctors, so we'd strongly advise you to search out a medical professional".

"However; the Rural Water User Association, Public Health Engineering Departments responsible to ensure the provision of safe water in targeted areas will be informed about the study outcome to take mitigation steps to safeguard the public health against arsenic complexities from contaminated drinking water." You may like to consider in advance what response you will give to villages where health issues are clear if they ask how they can mitigate at source. Are you, for example, taking on responsibility for communicating on the villages' behalf with the PHEDs or are you going to encourage them to lobby for action? In non-activist work we would generally advise taking an arms-length approach and simply stating that you will be feeding back to these bodies, rather than representing or encouraging anyone, but it is worth having an answer ready. If the team decides that they will take a more active role, please send the committee details of how this will be managed. Many thanks for the other confirmations.
Please notify the committee if you intend to make any amendments to the original research as submitted at date of this approval, including changes to recruitment methodology. All changes must receive ethical approval prior to implementation. The amendment form is available at http://ris.leeds.ac.uk/EthicsAmendment.

Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be audited. There is a checklist listing examples of documents to be kept which is available at http://ris.leeds.ac.uk/EthicsAudits. We welcome feedback on your experience of the ethical review process and suggestions for improvement. Please email any comments to ResearchEthics@leeds.ac.uk.

Yours sincerely

Jennifer Blaikie
Senior Research Ethics Administrator, Research & Innovation Service
On behalf of Dr Andrew Evans, Chair, AREA Faculty Research Ethics Committee

CC: Student's supervisor(s)

Contd. Appendix 3.1

Performance, Governance and Operations
Research & Innovation Service
Charles Thackrah Building
101 Clarendon Road
Leeds LS2 9LJ Tel: 0113 343 4873
Email: ResearchEthics@leeds.ac.uk

UNIVERSITY OF LEEDS

Hifza Johar Rasheed
School of Geography
University of Leeds
Leeds, LS2 9JT

Dear Hifza

AREA 14-005 – Amendment 1 – October 2017 - An Integrated Risk Assessment of Geogenic Arsenic Exposure and Attributable Health Risks

I am pleased to inform you that the amendment to the above research application as submitted by date of this email have been reviewed by the Chair of the AREA Faculty Research Ethics Committee and I can confirm a favourable ethical opinion as of the date of this email.

Please retain this email with your study file as evidence of approval.

The Chair noted the following:

- The strategies for consent seeking are appropriate and suitable for under 16 year olds via their parents
- In-country approval from Pakistan is in place

Please notify the committee if you intend to make any further amendments to the original research as submitted at date of this approval as all changes must receive ethical approval prior to implementation. The amendment form is available athttp://ris.leeds.ac.uk/EthicsAmendment. Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be audited. There is a checklist listing examples of documents to be kept which is available athttp://ris.leeds.ac.uk/EthicsAudits.

I hope the study continues to go well.

Best wishes
Rachel
On behalf of Dr Kahryn Hughes, Chair, AREA FREC

Rachel de Souza
Research Ethics & Governance Administrator
The Secretariat
Room 9.29, Level 9
Worsley Building, Clarendon Way
University of Leeds, LS2 9NL
Tel: 0113 3431642
r.e.desouza@leeds.ac.uk

Appendix 3.2: Ethical Approval from National Bioethics Committee Pakistan

National Bioethics Committee (NBC) Pakistan

Ref: No.4-87/14/NBC-150/RDC/ 3

Date: ~~June 25,2014~~ 01-07-14

Chairperson

Secretary, Cabinet Division
Government of Pakistan

Secretariat

Pakistan Medical Research Council

Members Ex-Officio

President
College of Physicians and surgeons of Pakistan

President
Pakistan Medical and Dental Council

Executive Director
Pakistan Medical Research Council (Member/Secretary)

WHO Country Representative

President
Supreme Court Bar Association

Surgeon General Pakistan Army

Director General Health, Punjab

Director General Health, Sindh

Director General Health, Khyber Pakhtoon Khawa

Director General Health, Balochistan

Director General Health, Gilgit Baltistan

Members

Prof. Dr. Zulfiqar A. Bhutta *(Chairman REC)*
Prof. Dr. Aasim Ahmad
Prof. Dr. Anis Ahmed
Dr. Anwar Nasim
Prof. Dr. Mohammad M. Amin
Prof. Dr. Farhat Moazzam
Dr. Maqbool H. Jafary
Dr. (Mrs) Anwar Aziz
Dr. Shaukat Ali Jawaid
Dr. Asmatullah
Prof Dr. S. Haroon Ahmad
Dr. Farid Khan
Prof. Dr. Baqi Durrani
Dr. Muhammad Zaheen

Ms Hifza Rasheed
Deputy Director
National Water Quality Laboratory (NWQL)
Pakistan Council of Research in Water Resources (PCRWR)
Khayaban-e-Johar Service Road South,
Sector H-8/1, Islamabad.

Subject: **Probabilistic Assessment of Geogenic Arsenic Exposure and Attributable Health Risks in Pakistan (NBC-150-AKU).**

Dear Ms Hifza Rasheed

I am pleased to inform you that the above mentioned project has been cleared by "Research Ethics Committee of National Bioethics Committee".

Kindly keep the National Bioethics Committee Secretariat updated with the progress of the project and submit the formal final report on completion.

Yours sincerely

(Prof Dr. Aasim Ahmad)
Chairman
NBC-Research Ethics Committee

NBC Secretariat:
Pakistan Medical Research Council, Shahrah-e-Jamhuriat, Off Constitution Avenue, Sector G-5/2, Islamabad
nbcpakistan.org.pk, www.pmrc.org.pk, e-mail: pmrc.rdc@gmail.com Tel: 92-51- 8207386, 9216793, 9205480, Fax 9216774, 9204559

Appendix 3.3. Preliminary information sheet

UNIVERSITY OF LEEDS

School of Geography
University of Leeds
Leeds LS2 9JT
0113 343 3373

PRELIMINARY INFORMATION SHEET

"Exposure Assessment of Arsenic through Water and Food in Arsenic Affected Areas"

Name of Research Student: Ms. Hifza Johar

Name of Research Supervisor: Dr. Rebecca Slack

Arsenic occurs naturally in many wells and aquifers, but at very high levels may lead to health problems. To investigate potential levels of arsenic in your village, our research team will take samples of your food and water. Therefore, the cooperation and support of you and your family member(s) for participation in this research study is highly desirable for undertaking the following major activities of this programme:

a) **Sampling of groundwater** from community source(s)/private source(s) being used by you and your family for drinking and cooking ***(Main Activity).***
b) **Sampling of wheat, raw and cooked rice** being consumed by you and your family member(s) ***(Main Activity).***
c) **Data collection** from you and your family member(s) by our team on 24 hours water and food in-take ***(Optional Activity: subject to the willingness of individual householders).***
d) **Sampling of hair, nail and urine** from you and your family member(s) ***(Optional Activity: subject to the willingness of individual householders).***

By testing water, food and biomarkers (hair, nail and urine) for arsenic, we will better understand what levels of arsenic exist in the natural environment and how this might be affecting the health and wellbeing of the village. Your cooperation and support for the main activities and optional activities described above is sought in order to provide information to the Government and Non-Government agencies regarding the types of work that might be required to further safeguard health.

Hifza Rasheed
PhD Student
School of Geography
University of Leeds, UK

Note: "All data will be kept entirely anonymized in any publications based on this work and the data ultimately destroyed when the work has been concluded"

Appendix 3.4. Detailed information sheet (Detailed)

School of Geography
University of Leeds
Leeds LS2 9JT
0113 343 3373

UNIVERSITY OF LEEDS

STUDY INFORMATION SHEET

Study Title: **Assessment of Geogenic Arsenic Exposure and Attributable Health Risks in Pakistan**

Name of Research Student: Ms. Hifza Rasheed
Name of Research Supervisor: Dr. Rebecca Slack
Name of Medical Doctor/health worker:_____
Name of Interviewer:_____

1. INTRODUCTION TO STUDY

As part of this research study, you are being invited to take part in an optional research study. 'Optional' means that you may refuse to take part in this study but still participate in the project looking at food and water. This study will include only people who choose to take part. Please take your time to make your decision.

Before agreeing to participate, it is important for you to understand all of the information related to this optional research study. Please ask the research student or study staff to explain any words in this document that you don't understand, and make sure that all your questions have been answered to your satisfaction before signing this consent form. Feel free to discuss the information in this document with your friends and family or your family doctor.

2. PURPOSE OF THIS OPTIONAL STUDY

If you agree to allow biomarker sampling, the researcher will study your samples and examine the level of arsenic and to compare it with the information to be collected on your food and water intake in 24 hours.

BIOMARKERS SAMPLING FOR RESEARCH

This optional study involves the collection of your hair, nail and urine samples for the testing of arsenic.

3. ANTICIPATED RESEARCH/USE OF THE SAMPLES AND STUDY DATA

This biomarker test will help researchers to understand how arsenic might be taken up by the body and, by comparing it to samples of food and water, where this arsenic comes from. After testing of for different types of arsenic (Total Arsenic, Arsenic III, V and monomethylarsonous acid and dimethylarsinic acid), your samples will be destroyed.

4. WHAT DOES PARTICIPATION IN THIS OPTIONAL STUDY MEAN?

If you agree to take part, the following samples will be obtained from you:

1	**Urine**	**2 oz bottle.**
2	Nail	From big toe of foot (1g)
3	Hair	1g of hair to be cut near scalp

If you agree to donate your samples, we collect from you at your convenience.

5. WHAT WILL HAPPEN TO YOUR SAMPLES AND STUDY DATA?

Your biomarkers samples will be sent to the National Water Quality Laboratory, Pakistan Council of Research in Water Resources, Govt. of Pakistan, Islamabad where each sample will be preserved. These samples will be shipped to the Applied Speciation and Consulting, LLC, 18804 Northcreek Parkway, WA, USA to be analysed. These samples will be destroyed after testing. The data will arising from the laboratory analysis will be examined at the University of Leeds, Leeds, UK.

6. WHO WILL HAVE ACCESS TO YOUR SAMPLES AND STUDY DATA?

Your samples and study data will be used only by the research student who is registered at the University of Leeds: it will not be sold and you will not be identified from your study data.

7. WHAT ARE THE RISKS OF THIS OPTIONAL STUDY?

There is no health risks associated with this study.

8. ARE THERE BENEFITS TO PARTICIPATING IN THIS OPTIONAL STUDY?

The data are being collected as part of a three year research project. The research findings (but not the individual results) may help to motivate the local water supply authorities to adopt initiatives to provide safe water to the local communities.

9. WHAT ABOUT CONFIDENTIALITY?

To protect your identity and privacy, your samples will be labelled with a unique study number or 'code' before they are sent to the study sponsor, but not with any personal identifiers such as your name or initials. The code linking your personal identifiers to the sample will be kept by the researcher in a secure and confidential location. As such, your samples will be anonymised.

The study researcher may include specific information with the sample (such as your age, your gender, or certain clinical, pathological or demographic data, etc.); however, this information is unlikely to allow you to be identified or retraced. You should know that the removal of some or all of your personal information from the study data is known as de-identification. This de-identification of the study data is intended to protect your privacy and the chances of being re-identification are very small.
Qualified representatives of the testing laboratory will only receive samples with the unique study number or 'sample code' for laboratory analysis. Regulatory authorities, such as National Bioethics Committee of Pakistan may also wish to check that the study has been done properly, and will also have direct access to your personal information.

Except as expressly stated in this section, all of the information provided in the main study consent form about confidentiality and direct access to your personal information applies to this optional study and biomarker information: water and food data cannot be used to identify you. Unless otherwise, it becomes important for your health, the test result of your hair, nail and urine may also be shared with your medical doctor for possible treatment subject to your consent for this purpose.

10. WILL YOU RECEIVE ANY COMPENSATION PARTICIPATING IN THIS OPTIONAL STUDY?

You will not receive any compensation to participate in this study.

11. WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

Taking part in this optional sample study is entirely your choice. You can choose not to take part, or you can change your mind at any time for any reason. Your decision will not affect your medical care or your relationship with the study researcher in any way. You may refuse to take part in this study. There will be no violation of Children Rights (following the Pakistan's Protection of Children Act, 2006). Although no risks are involved in this study, however; the children's parents can approach the local Child Protection Officer to obtain protective measures to the child-in-need of care as a result of any violence on him.

If you take part in this optional sample study and then decide that you no longer want your samples to be used, you can contact and inform the research student about your refusal to participate or disposal of your samples at any time and at any stage of sampling, samples transportation and samples processing.

Ms. Hifza Rasheed
Research Student, School of Geography, University of Leeds
Telephone No. Pakistan: 0092-323-5251219
UK: 0044(0)7835567726
Email: pcrwr2005@yahoo.com, gyhj@leeds.ac.uk

If you withdraw your consent **before** your sample is sent to the testing laboratory, the study researcher will arrange to have these destroyed. If you withdraw your consent **after** your sample has been sent to the **testing laboratory,** the unused samples will be destroyed. The study sponsor will not make any results available to you, any insurance company, your employer, your family, the study doctor, or any other physician who treats you now or in the future.

12. TERMINATION OF STUDY?

You will be informed about any significant new findings developed during the course of this study that may relate to or influence your willingness to continue participation. As a result, if you decide to discontinue your participation in the study you can contact following person to inform about your decision:

Ms. Hifza Rasheed
Research Student, School of Geography, University of Leeds
Telephone No. Pakistan: 0092-323-5251219
UK: 0044(0)7835567726
Email: pcrwr2005@yahoo.com, gyhj@leeds.ac.uk

Note: Your participation in the study may also be terminated by the investigator without your consent in case of shortage of funding and samples storage and transportation facilities.

13. EMERGENCY CONTACT?

Following contact persons will answer your any further question related to the study.

Ms. Hifza Rasheed
Research Student, School of Geography, University of Leeds
Telephone No. Pakistan: 0092-323-5251219; UK: 0044(0)7835567726
Email: pcrwr2005@yahoo.com, gyhj@leeds.ac.uk

Dr Rebecca Slack
water@leeds coordinator
School of Geography
University of Leeds
Leeds, LS2 9JT
Telephone No.0044 (0)1133433373

Appendix 3.5. Informed consent

Part-A: Consent of study Participant		Form No.	
Date		Village	
Person Name		Person ID	
Gender		Age	
House ID		Name of the Local Medical Doctor/Physician	

Research Student

I am Hifza Johar, a Ph.D. researcher at School of Geography, University of Leads to undertake this study focused on your village:

_____ _____

Research Student's Signature Date

Person obtaining informed consent and data collection:

My signature below signifies that I have explained the nature and purpose of the study and the risks involved to the study participant, and I have answered all questions to the best of my ability. I am willing to collect data of human subjects for daily water and food intake, prevailing diseases and samples of hair, nail and urine. I am explained the risks and benefits involved in this field work.

Name of Person Obtaining Informed Consent	Signature of Person Obtaining Informed Consent	Date

Consent to participate in this research study (by participant)

My signature on this consent form means that:

This optional study has been explained to me, I have been given the chance to discuss it and ask questions. All of my questions have been answered to my satisfaction	**Yes** □	**No** □
I have read each page of this form	**Yes** □	**No** □
I am aware of the risks to me of participating in this optional study	**Yes** □	**No** □
I agree to allow access to my personal food and health examination information as explained in this form	**Yes** □	**No** □
I agree to allow collection of my hair, nails, urine and blood samples and food and water intake data of 24 hrs for the research purposes explained in this form	**Yes** □	**No** □
I voluntarily consent to take part in this optional study	**Yes** □	**No** □
I allow taking my picture and its use for any academic publication	**Yes** □	**No** □
I allow sharing of my test results with my family under special circumstances	**Yes** □	**No** □

Name of Participant	Signature of Participant	Date

I voluntarily consent that biomarkers samples from my children may be take with his/her consent

Name of Parent(s) of <16 age participant	Signature of Parent(s)	Date

Whom do you call if you have questions?

If you have questions about donating your samples, any study-related injury, or your rights as a study participant, you may contact the Ms. Hifza Rasheed, Research Student, School of Geography, University of Leeds, Telephone No. 0092-323-5251219. Email: gyhj@leeds.ac.uk, pcrwr2005@yahoo.com.

Appendices-Chapter 4

Appendix 4.1. Survey Questionnaire (Interview based)

Part-B: Demographic Information of Household			
Date		District	
City		Village	
House ID		No. of persons in House	
IDs of Family members/persons			
Name	Person ID	Name	Person ID

Part-C: 24 hrs Water and Beverage Intake Record			
Date		Village	
House-ID		Person ID	

Sr. No	Period	Time	Water intake (No. of Glass)	Tea (No. of Cups)	Juices/cold drinks etc.(No. of Glass)
1.	**Morning**	5:00-6:00 am			
2.		6:00-7:00 am			
3.		7:00-8:00 am			
4.		8:00-9:00 am			
5.		9:00-10:00 am			
6.		10:00-11:00 am			
7.	**Noon**	11:00-12:00 am			
8.		12am-1:00 pm			
9.		1:00-2:00 pm			
10.		2:00-3:00 pm			
11.	**Afternoon**	3:00-4:00 pm			
12.		4:00-5:00 pm			
13.		5:00-6:00 pm			
14.	**Evening**	6:00-7:00 pm			
15.		7:00-8:00 pm			
16.	**Night**	8:00-9:00 pm			
17.		9:00-10:00 pm			
18.		10:00-11:00 pm			
19.		11:00-12:00 pm			
20.		12:00-1:00 pm			
21.		1:00-2:00 am			
22.		2:00-3:00 am			
23.		3:00-4:00 am			
24.		4:00-5:00 am			

Standard size of glass equivalent to approx. 300 ml.

Part-D: 24 hrs Food Intake Record			
Date		Village	
House-ID		Person ID	
Body weight			

Introduction:

This interview is to enable us to find out what you have eaten during 24 hrs. What you need to do is to record-all that you have eaten. This will need to be recorded by you or with help of team member.

Sr. No	Timings	Time	Food Type	Unit	Qty Taken	Source of Food
1.	**Morning**	5:00-11:00 am	□ Chappati			
2.			□ Baked bread			
3.			□ Rice			
4.			□ Egg			
5.			□ Butter			
6.			□ Milk			
7.			□ Cream			
8.			□ Jam			
9.			□ Rusks			
10.			□ Cereals			
11.			□ Desserts			
12.			□ Any other_____			
13.	**Noon**	11:00 am	□ Chappati			
14.		03:00 pm	□ Vegetables_____			
15.			□ Fruit_____			
16.			□ Pulses_____			
17.			□ Mutton			
18.			□ Beef			
19.			□ Chicken			
20.			□ Fish			
21.			□ Salads			
22.			□ Rice			
23.			□ Desserts			
24.	**Afternoon**	3:00-6:00 pm	□ Snacks_____			
25.			□ Sandwiches			
26.			□ Biscuits			
27.			□ Rusks			
28.			□ Other_____			
29.	**Evening**	6:00-8:00 pm	□ Fruits_____			
30.			□ Snacks_____			
31.			□ Other_____			
32.	**Night**	8:00 pm 50:00 am	□ Chappati			
33.			□ Vegetables_____			
34.			□ Fruit_____			
35.			□ Pulses_____			
36.			□ Mutton			

Sr. No	Timings	Time	Food Type	Unit	Qty Taken	Source of Food
37.			□ Beef			
38.			□ Chicken			
39.			□ Fish			
40.			□ Salads			
41.			□ Rice			
42.			□ Desserts			
43.			□ Other_____			

Source of Food	1	2	3	4	5
	Home made	Restaurant/cafeteria/fast food shop	Food stall/hawker	Food store	Work place tuck shop
	6	7	8	9	10
	Day care	Friend/relative's home	Party/BBQ/Banquet/ special event	School/college tuck shop	Other (specify)

Appendix 4.2: Water and food intake calculation formulae

Equation No.	Food item	Unit and Eqv weight* (g)*	Water used* (g)	Weight per serving	Volume of water (ml)	*Equation used to calculate water (L person $^{-1}$ day^{-1}) or food intake (g person $^{-1}$ day^{-1})*
(1)	Tea, black, brewed, prepared with tap water (without milk)	1 cup (237 g)	236.29	120-200 ml	249.48	$WI_{tea} = \text{No of cups consumed per day} \times \text{ml of water per cup} / 1000$
(2)	Whole milk	1 cup (245 g)	215.38	5-10 ml (added in tea)	4.4-8.8	-
(3)	Fermented dairy drink (Lassi)	1 glass	96.2%**	250 ml	240	$WI_{lassi} = \text{No. of glass consumed per day} \times 240 \text{ ml of water} / 1000$
(4)	Rice, white, medium-grain, cooked	1 cup (186 g)	127.61 (69%)	300-414 g	206-284	$WI_{cooked\ rice} = \text{cooked rice intake in gm} \times 0.69 / 1000$
(5)	Red and White, Lentil Soup, condensed	1 cup (252 g)	179.42	150 g	107	$WI_{pulses} = \text{No. of servings (150 g)} \times \text{ml of water (107 ml)}/1000$
(6)	Bread, Chapatti or Roti, plain, commercially prepared	1 piece (68 g)	22.44	80-90 g (Av: 85 g)	28	$WI_{chapatti} = \text{No. of units consumed (85 g)} \times 28 \text{ ml of water}/1000$
(7)	Water intake from direct sources	-	-	-	-	$W_{direct} = \text{size of glass } (200 - 250 \text{ ml}) \times \text{No. of glass per day} / 1000$
(8)	Water intake from indirect sources	-	-	-	-	$TW_{indirect} = WI_{tea+lassi+cooked\ rice+pulses+chapatti}$
(9)	Total water intake	-	-	-	-	$WI_{total} = TW_{direct} + TW_{indirect}$
(10)	Total daily intake of food (TDFI)	-	-	-	-	$TDIF = \text{Weight offood measured on plate/bowl} \times \text{No. of servings per day}$

Whereas: WI= Water intake (L person $^{-1}$ day^{-1})

***** Standard values recommended by Standard Reference Release-27, National Nutrient Database of United States Department of Agriculture (USDA) (Agricultural Research Service, 2014)

**Lassi containing 96.2% water (Padghan et al., 2015)

Appendix 4.3: Description of study area participants

Parameter		unit	Villages						overall
			Chak-46/12-L	Chak-48/12-l	Chak 49/12-l	Basti Balochan	Badarpur	Basti Kotla Arab	
Households reported by PBS		n	447	412	522	260	395	319	1776
Average household size		n	7	7	7	7	8	8	29
Population reported by PBS		n	3,195	3,037	3,986	2036	3,393	2345	15647
Male population		n	1,599	1,559	2,071	1,006	1,714	1210	7949
Female population		n	1,596	1,478	1,915	1,030	1,679	1135	7698
Literacy ratio		%	34.1	53.7	59.1	24	43.4	23	14
Households willing to participate in the study		n	64	45	50	26	26	29	240
Sampled houses		%	15	11	10	10	10	15	14
Total participants		n	121	54	75	44	34	70	398
Men		n	79	49	59	14	20	28	249
Age range	< 16	n	19	4	6	6	0	8	43
	≥16	n	60	45	53	8	20	20	206
Body weight range (kg)	< 35 kg	n	19	0	13	25	.	6	--
	≥ 35 kg	n	69	52	55	32	51	48	--
Women									
Age range	< 16	n	7	2	2	2	1	9	23
	≥16	n	35	3	14	28	13	33	126
Body weight range (kg)	< 35 kg	n	20	1	13	16	0	19	--
	≥ 35 kg	n	68	14	41	30	38	36	--

Source: Pakistan Bureau of Statistics (PBS)

Appendix 4.4: Food and beverages sources contributing to indirect water intake (L person $^{-1}$ day^{-1})

Villages	Age groups	Indirect water intake				Sources				
						Wheat Chapatti	Rice	Pulses	Tea	Lassi
		Min	Max	Mean	SD	Mean	Mean	Mean	Mean	Mean
Chak-46/12-L	Age < 16	0.1	1.0	0.4	0.2	0.1	0.2	0.1	0.1	0.3
	Age > 16	0.2	2.0	0.8	0.4	0.1	0.4	0.2	0.2	0.5
Chak-48/12-I	Age < 16	0.3	1.9	1.1	0.6	0.1	0.2	0.2	0.3	0.9
	Age > 16	0.4	2.2	1.1	0.5	0.1	0.3	0.2	0.3	0.8
Chak 49/12-I	Age < 16	0.1	1.2	0.4	0.3	0.1	0.1	0.1	0.1	1.0
	Age > 16	0.3	2.3	0.9	0.4	0.1	0.4	0.2	0.4	0.5
Basti Balochan	Age < 16	0.1	1.0	0.6	0.3	0.1	0.2	0.1	0.2	0.3
	Age > 16	0.3	1.4	0.7	0.3	0.1	0.2	0.2	0.2	0.4
Badarpur	Age < 16	0.3	0.3	0.3	0.0	0.1	0.0	0.1	0.1	0.0
	Age > 16	0.3	2.4	1.0	0.5	0.1	0.4	0.2	0.2	0.7
Kotla Arab	Age < 16	0.1	1.4	0.6	0.4	0.1	0.1	0.1	0.2	0.7
	Age > 16	0.3	1.9	1.0	0.5	0.1	0.3	0.2	0.2	0.7
Total	Age < 16	0.1	1.9	0.6	0.4	0.1	0.2	0.1	0.2	0.5
	Age > 16	0.2	2.4	0.9	0.4	0.1	0.4	0.2	0.3	0.6
	Overall	0.1	2.4	0.8	0.4	0.1	0.3	0.2	0.2	0.6

Appendix 4.5: Village wise average daily water intake (L day^{-1} person^{-1}) of the study population

Village	Sex	Age groups (years)	Direct Water Intake (L person^{-1} day^{-1})		In-direct Water Intake (L person^{-1} day^{-1})		Total Water Intake (L person^{-1} day^{-1})		Total Water Intake (L kg^{-1} day^{-1})	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chak-46/12-L	Children	3-6	1.6	0.2	0.1	0.0	1.8	0.2	0.1	0.0
		6-16	2.3	0.5	0.5	0.2	2.8	0.5	0.1	0.0
		Overall < 16	2.3	0.5	0.4	0.2	2.7	0.6	0.1	0.0
	Male	≥ 16	3.0	0.8	0.9	0.4	3.9	0.9	0.1	0.0
	Female	≥ 16	2.5	0.4	0.7	0.3	3.2	0.6	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	2.8	0.7	0.8	0.4	3.6	0.8	0.1	0.0
	Average intake	All participants	2.7	0.7	0.7	0.4	3.4	0.9	0.1	0.0
Chak-48/12-I	Children	3-6	.	.	.	.	.	.	.	.
		6-16	2.6	0.3	1.1	0.6	3.8	0.6	0.1	0.1
		Overall < 16	2.6	0.3	1.1	0.6	3.8	.6	0.1	0.1
	Male	≥ 16	2.9	0.9	1.1	0.6	4.0	1.2	0.1	0.0
	Female	≥ 16	2.7	0.4	1.0	0.2	3.8	0.5	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	2.8	0.9	1.1	0.5	3.9	1.2	0.1	0.0
	Average intake	All participants	2.8	0.9	1.1	0.5	3.9	1.1	0.1	0.0
Chak 49/12-I	Children	3-6	1.8	0.8	0.7	0.7	2.5	1.6	0.2	0.0
		6-16	2.4	0.7	0.3	0.1	2.7	0.8	0.1	0.0
		Overall < 16	2.3	0.8	0.4	0.3	2.7	0.9	0.1	0.0
	Male	≥ 16	2.7	0.9	0.9	0.4	3.6	0.9	0.1	0.0
	Female	≥ 16	2.0	0.3	0.8	0.3	2.8	0.5	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	2.5	0.8	0.9	0.4	3.4	0.9	0.1	0.0
	Average intake	All participants	2.5	0.8	0.8	0.4	3.3	0.9	0.1	0.0
Basti Balochan	Children	3-6	1.2	0.0	0.1	0.0	1.3	0.0	0.1	0.0
		6-16	2.5	0.6	0.7	0.2	3.1	0.7	0.1	0.0
		Overall < 16	2.3	0.7	0.6	0.3	2.9	0.9	0.1	0.0

Village	Sex	Age groups (years)	Direct Water Intake (L person^{-1} day^{-1})		In-direct Water Intake (L person^{-1} day^{-1})		Total Water Intake (L person^{-1} day^{-1})		Total Water Intake (L kg^{-1} day^{-1})	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Male	≥ 16	3.4	0.5	0.6	0.2	4.0	0.5	0.1	0.0
	Female	≥ 16	2.4	0.4	0.7	0.3	3.1	0.4	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	2.7	0.6	0.7	0.3	3.3	0.6	0.1	0.0
	Average intake	All participants	2.6	0.6	0.6	0.3	3.2	0.7	0.1	0.0
Badarpur	Children	3-6	.	.	.	.	.	.	.	.
		6-16	2.4	0.0	0.3	0.0	2.7	0.0	0.1	0.0
		Overall < 16	2.4	0.0	0.3	0.0	2.7	0.0	0.1	0.0
	Male	≥ 16	3.2	0.4	1.0	0.5	4.2	0.6	0.1	0.0
	Female	≥ 16	3.0	0.5	0.9	0.6	3.9	0.7	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	3.1	0.4	1.0	0.5	4.1	0.7	0.1	0.0
	Average intake	All participants	3.1	0.4	0.9	0.5	4.0	0.7	0.1	0.0
Kotla Arab	Children	3-6	.	.	.	.	.	.	.	.
		6-16	2.1	0.4	0.6	0.4	2.8	0.6	0.1	0.0
		Overall < 16	2.1	0.4	0.6	0.4	2.8	0.6	0.1	0.0
	Male	≥ 16	2.9	1.1	1.1	0.5	4.0	1.3	0.1	0.0
	Female	≥ 16	2.1	0.7	0.9	0.4	3.0	0.8	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	2.4	0.9	1.0	0.5	3.4	1.2	0.1	0.0
	Average intake	All participants	2.4	0.8	0.9	0.5	3.2	1.1	0.1	0.0
Overall (All villages)	Children (both sex)	<16	2.3	0.5	0.6	0.4	2.8	0.7	0.1	0.0
	Male	≥ 16	2.9	0.9	1.0	0.5	3.9	1.0	0.1	0.0
	Female	≥ 16	2.4	0.5	0.8	0.4	3.2	0.7	0.1	0.0
	Average intake (irrespective of sex)	≥ 16	2.7	0.8	0.9	0.4	3.6	0.9	0.1	0.0
	Average intake	All participants	2.6	0.8	0.8	0.4	3.5	1.0	0.1	0.0

Appendix 4.6: Reported water intake values in different countries

Country	Male			Female			All adults			Intake Type	Reference
	n	age range	L day^{-1}	n	age range	L day^{-1}	n	age range	L day^{-1}		
Australia	ND	19+	3.4	ND	19+	2.8	ND	19+	3.1	water, hot and cold beverage intake	Commonwealth scientific and industrial research organisation and University of South Australia (2008)
Australia	ND	ND	ND	ND	ND	ND	ND	ND	2	water	NHMRC (2011)
Canada	ND	ND	ND	ND	ND	ND	8,916	ND	1.2	water	Roche et al. (2012)
Canada	37	ND	ND	88	ND	ND	125	20 to 64	1.6	Water, beverages and liquid food	Levallois et al. (1998)
Canada	ND	ND	ND	ND	ND	ND	4532	ND	1	water	Jones et al. (2007)
USA	ND	>19	3	ND	ND	3	4,112	>19	3.17	total fluids intake	Kant et al. (2009)
USA	7614	ND	ND	8088	ND	ND	15702	20 to ≥71	3.5	water, hot and cold beverage intake	Drewnowski et al. (2013)
USA-Winters	ND	ND	ND	ND	ND	ND	2458	ND	0.983	water	Barraj et al. (2009)
USA-summers	ND	ND	ND	ND	ND	ND	1740	ND	1.1	water	Barraj et al. (2009)
USA	ND	ND	ND	ND	ND	ND	20,000	<1 month to >65 years	2.6	water	Kahn and Stralka (2009)
USA	11,888	<1 to >65	2.261	14193	<1 to >65	1.919	26081	20 to 65	2.07	direct and indirect water intake (beverages and food)	Ershow and Cantor (1989)

Country	Male			Female			All adults			Intake Type	Reference
	n	age range	L day^{-1}	n	age range	L day^{-1}	n	age range	L day^{-1}		
USA	ND	ND	ND	ND	ND	ND	ND	≥21	2.5	water	United States Environmental Protection Agency (2011)
USA	ND	ND	1.3	ND	ND	1.18	20,261	<1 to >20	1	water	US Environmental Protection Agency (2004)
Mexico	574	ND	1.77	ND	ND	1.84	1498	38.6	1.81	total fluids intake	Martinez (2014)
Mexico	ND	18 to ≥50	ND	ND	ND	ND	80	20–65	1.81	water	Del Razo et al. (2002)
Brazil	941	18 to ≥50	2.34	983	18 to ≥50	2.1	1924	ND	2.22	water, hot and cold beverage intake	Guelinckx et al. (2015)
Argentina	241	18 to ≥50	2.32	266	18 to ≥50	2.29	507	ND	2.3	water, hot and cold beverage intake	Guelinckx et al. (2015)
UK	1,758	1 to >55	1.07	1,800	1 to>55	1.87	3,564	1 to >55	1.59	water, hot and cold beverage intake	Hopkin and Ellis (1980)
UK	371	ND	2.24	526	ND	2.37	897	ND	2.32	total fluids intake	Gandy (2015)
Spain	630	18 to ≥50	1.94	610	18 to ≥50	1.87	1240	ND	1.9	total fluids intake	Ferreira-Pêgo et al. (2014)
France	ND	ND	ND	ND	ND	ND	1361	20 to 54	1.31	water, hot and cold beverage intake	Bellisle et al. (2010)
France	804	18 to ≥50	1.55	730	18 to ≥50	1.57	1534	ND	1.56	water, hot and cold beverage intake	Guelinckx et al. (2015)
Poland	517	18 to ≥50	1.7	545	18 to ≥50	1.57	1062	ND	1.64	water, hot and cold beverage intake	Guelinckx et al. (2015)
Turkey	488	18 to ≥50	2.15	473	18 to ≥50	2.17	961	ND	2.21	water, hot and cold beverage intake	Guelinckx et al. (2015)
France	ND	ND	ND	ND	ND	ND	831	20 to 54	2	water, hot and cold beverage intake	Bellisle et al. (2010)
Germany	639	>17	3	889	>17	ND	1528	ND	ND	direct and indirect water intake (beverages and food)	Manz et al. (2012)
Germany	856	18 to ≥50	2.51	1012	18 to ≥50	2.45	1868	ND	2.47	water, hot and cold beverage intake	Guelinckx et al. (2015)
Sweden	585	ND	2	625	ND	2	1210	ND	ND	water, hot and cold beverage intake	Shirreffs (2012)

Country	Male			Female			All adults			Intake Type	Reference
	n	age range	L day^{-1}	n	age range	L day^{-1}	n	age range	L day^{-1}		
Sweden	ND	ND	ND	ND	ND	ND	10957	ND	1.86	water and hot beverages	Westrell et al. (2006)
Netherlands	1252	22 to 50	3	1472	22-50	2	2724	ND	1.5	water	European Food Safety Agency and Allergies (2010)
Indonesia	444	18 to ≥50	2.33	922	18 to ≥50	2.26	1366	ND	2.28	water, hot and cold beverage intake	Guelinckx et al. (2015)
Malaysia	ND	ND	102	103	ND	ND	ND	ND	ND	water	Azlan et al. (2012)
Pakistan	ND	ND	102	103	ND	ND	ND	ND	4	water	Arain et al. (2009)
India	ND	ND	4	ND	ND	3	9	ND	ND	water	Chowdhury et al. (2000)
India	219	≥15 years	6.1	204	≥15 years	4.84	423	7 months to 90 years	4.92	direct and indirect water intake (beverages and food)	Hossain et al. (2013)
India	50	19-68	4.8	50	19-68	3.3	100	19-68	4.5	Water, mixed drinks, rice and pulses	Pokkamthanam et al. (2011)
Bangladesh	127	>14	3.89	323	>14	3.02	ND	0 to >65	ND	water	Khan et al. (2009)
Bangladesh	ND	ND	73.97 ml kg^{-1} day^{-1}	ND	ND	72.07 ml kg^{-1} day^{-1}	640	15 to ≥45	3.53	water	Milton et al. (2006)
Bangladesh	28	16 to 80	3.1	23	20 to 70	2.9	77	6 to 80	3	water	Ohno et al. (2007)
Bangladesh	9	>20	3	9	>20	3	38	20 to 53	3	water	Watanabe et al. (2004)
Bangladesh	113	16 to 73	3.1	108	14 to 65	2.6	232	14 to 65	ND	water	Mondal et al. (2010)
Bangladesh	5042	ND	2.9	6704	ND	3.1	ND	ND	ND	water	Ahsan et al. (2006)
Bangladesh	ND	ND	ND	ND	ND	ND	936	20 to 65	2.55	water	Kile et al. (2007)
Pakistan	249	3 to 80	3.70	149	4 to 80	3.11	398	3 to 80	3.50	direct and indirect water intake (beverages and food)	Present study
Iran	283	ND	1.92	289	ND	1.92	572	ND	1.92	total fluids intake	Abdollahi et al. (2013)
China	733	ND	1.78	733	ND	1.75	1466	ND	1.76	total fluids intake	Ma et al. (2012)
Japan	698	18 to ≥50	1.47	683	18 to ≥50	1.52	1381	ND	1.5	water, hot and cold beverage intake	Guelinckx et al. (2015)
Taiwan	ND	ND	1.5	ND	ND	1	ND	ND	1.2	water	Liang et al. (2016)

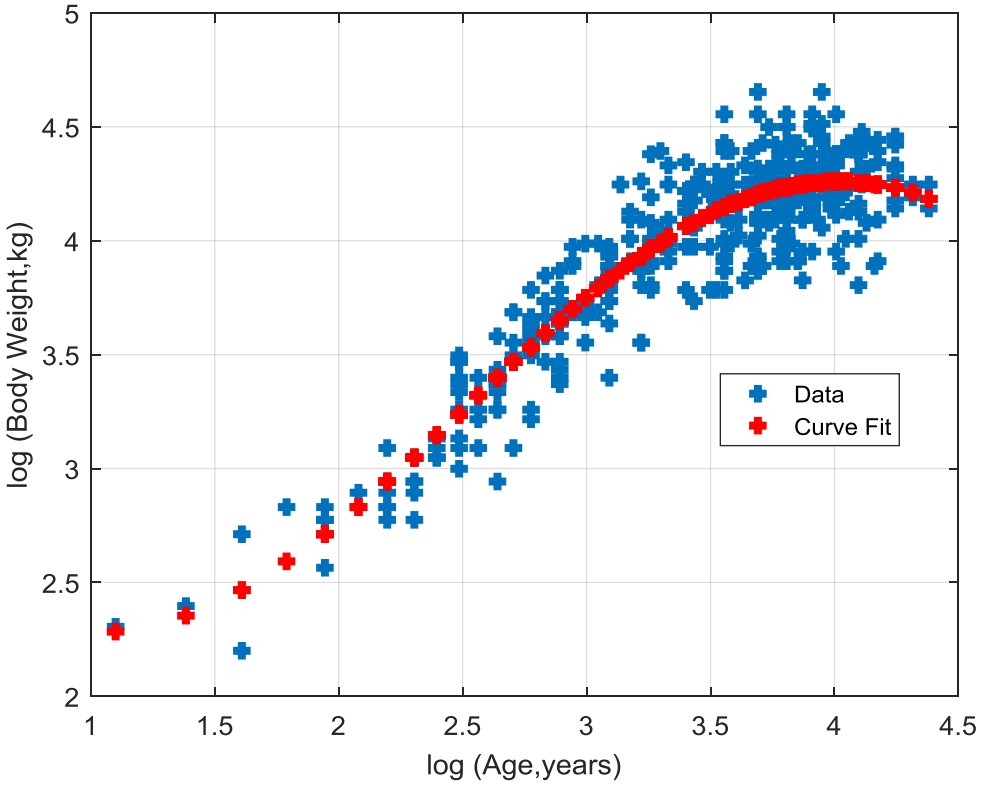
n: No. of samples, ND: No data

Appendix 4.7: Average daily rice, wheat and vegetables intake (g day^{-1} person^{-1}) reported in different countries/regions

Country	Food item	Consumption g day^{-1}				Reference
		Children	Men	Women	Mean	
India	Rice (cooked)				450	Signes et al. (2008)
India	Rice (cooked)	400 (around 10 years of age)	750	750	713	Roychowdhury et al. (2002)
China	Rice (cooked)	210			370	Song et al. (2015)
Sweden	Rice (cooked)				44	Sand et al. (2015)
Korea	Rice (cooked)		236.8	187	212	Cha et al. (2012)
Thailand	Rice (cooked)				>200	Saipan and Ruangwises (2009)
Bangladesh	Rice (cooked)	862	1789	1522	1391	Khan et al. (2009)
Bangladesh	Rice (cooked)				1782	Melkonian et al. (2013)
Bangladesh	Rice (cooked)		523	300		Watanabe et al. (2004)
Bangladesh	Rice (raw)				400	Duxbury et al. (2003)
Bangladesh	Rice (raw)				420	Meharg and Rahman (2003)
Cambodia	Rice (cooked)				522	Gilbert et al. (2015)
Bangladesh	Rice (cooked)		776	553	665	Ohno et al. (2007)
Pakistan	Rice(cooked)	253	576	463	372	Present study
Pakistan	Rice(cooked)			259		Aga Khan University et al. (2011)
Finland	Rice(cooked)	24			83	Rintala et al. (2014)
USA	Rice (Raw)	5		11	17	U.S. Food and Drug Administration (2016)
USA	Rice (Cooked)	88			172.6	U.S. Food and Drug Administration (2016)
USA	Rice (Raw)	17				Batres-Marquez et al. (2009)
USA	Rice (cooked)				334	Smiciklas-Wright et al. (2003)
Europe	Rice (cooked)				175	European Food Safety (2014)
Europe	*Rice				12	World Health Organization (2003)
Africa	*Rice				103	World Health Organization (2003)
Middle East	*Rice				48	World Health Organization (2003)

Country	Food item	Consumption g day^{-1}				Reference
		Children	Men	Women	Mean	
Far East	*Rice				279	World Health Organization (2003)
Latin America	*Rice				87	World Health Organization (2003)
Cambodia, Indonesia, Lao People's Democratic Republic, Mayanmar and Vietnam	Rice (raw polished rice)				>400	Kennedy et al. (2002)
Cambodia	Rice (cooked)				522	Gilbert et al. (2015)
Vietnam	Rice (cooked)				460	Agusa et al. (2006)
Bangladesh	Wheat		179	131		Watanabe et al. (2004)
China		13			44	Zeng et al. (2015)
Europe					182	Food and Agriculture Organization (2013)
USA					48	U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015)
Pakistan					250	Mahmood et al. (2014)
Pakistan				306		Aga Khan University et al. (2011)
Pakistan		222	426	358	402	Present study
Cambodia	Vegetables				417-656	Wang et al. (2013)
Republic of Croatia					275	Sapunar-Postružnik et al. (1996)
Chile					327	Muñoz et al. (2005)
Denmark					376	Helgesen and Larsen (1998)
India					400-500	Samal et al. (2011) Roychowdhury et al. (2003)
Pakistan					100	Arain et al. (2009)
Pakistan		103	187	181	170	Present study

*raw or cooked status is not mentioned in the WHO/FSF/FOS/97.7.



Appendix 4.8: Age and body weight of participant's-linear regression

General model Fourier Fit: (Goodness of Fit R-sq 0.85)

f(x) = a0 + a1*cos(x*w) + b1*sin(x*w)

Coefficients (with 95% confidence bounds):
a0 = 3.269 (3.139, 3.399)
a1 = -0.4815 (-0.9603, -0.002601)
b1 = -0.8643 (-0.9992, -0.7294)
w = 1.047 (0.9079, 1.187)
x = ge (in log)
f(x) = Body Weight (in log)

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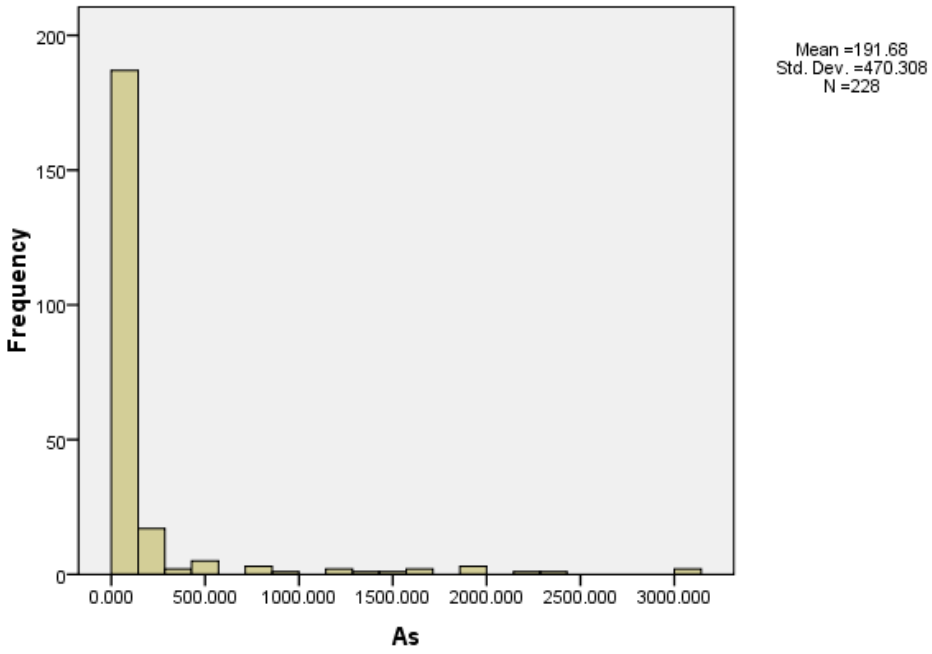
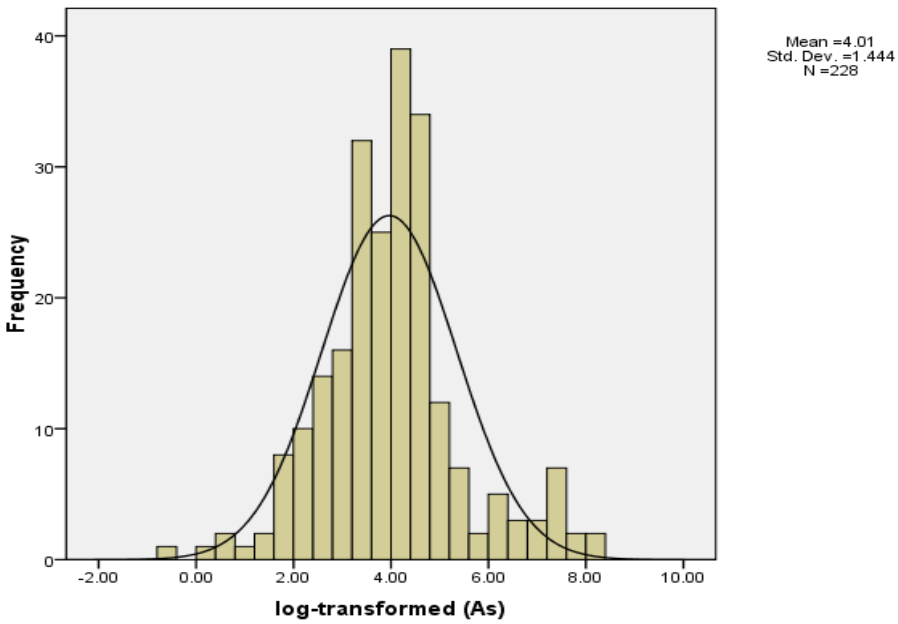


Appendix 5.1. Distribution frequency of total arsenic (original data) concentrations in ground water



Appendix 5.2. Distribution frequency of total arsenic (log transformed data) concentrations in ground water

Appendix 5.3. Summary statistics of log transformed total arsenic and inorganic arsenic species (µg L^{-1}) in groundwater samples (n = 228)

Analyte	Statistics	Chak-46/12-L	Chak-48/12-I	Chak 49/12-I	Basti Balochan	Badarpur	Basti Kotla Arab	Overall
No of samples	n	57	45	50	31	16	29	228
As (Total)	AM	3.91	4.98	3.89	3.16	6.98	2.22	4.01
	SD	0.86	1.06	0.66	0.37	1.10	1.1	1.44
	GM	4.85	4.85	3.83	3.11	6.88	*	*
	GSD	2.35	2.97	1.93	1.58	2.98	3.02	4.24
	95% CI LB	3.68	4.65	3.71	3.02	6.40	1.80	3.82
	95% CI UB	4.13	5.31	4.08	3.31	7.56	2.64	4.20
	Log-Median	4.18	5.04	4.12	3.25	7.42	2.43	4.05
	Minimum	1.27	2.14	1.96	2.11	3.78	0.73	0.73
	Maximum	5.43	7.24	4.56	3.63	8.04	3.94	8.04
As^{+5}	AM	3.90	3.95	3.54	2.95	7.09	2.26	3.89
	SD	0.89	1.15	0.9	0.42	1.09	1.1	1.53
	GM	3.75	4.7	3.4	2.88	6.99	*	*
	GSD	2.429	3.159	2.449	1.651	2.984	3.717	4.617
	95% CI LB	3.67	4.5	3.29	2.78	6.51	1.76	3.69
	95% CI UB	4.14	5.19	3.8	3.12	7.67	2.76	4.09
	Log-Median	4.16	4.16	3.83	3.05	7.53	2.53	3.95
	Minimum	0.88	2.04	1.1	1.62	3.87	-2.21	-2.21
	Maximum	5.4	7.27	4.66	3.39	8.14	4.14	8.14
As^{+3}	AM	-0.96	-0.21	1.35	-0.18	-0.36	-0.68	-0.12
	SD	0.14	1.34	2.00	0.86	0.7	0.58	1.45
	GM	*	*	*	*	*	*	*
	95% CI LB	-1.00	-0.61	0.79	-0.52	-0.73	-0.90	-0.31
	95% CI UB	-0.92	0.19	1.92	0.16	0.02	-0.46	0.07
	Log-Median	-0.99	-0.99	0.99	-0.49	-0.51	-0.99	-0.99
	Minimum	-0.99	-0.99	-0.99	-0.99	-0.99	-0.99	-0.99
	Maximum	-0.04	4.05	4.61	1.57	1.18	0.82	4.61

n: Number of samples; AM: Arithmetic mean; SD: Arithmetic standard deviation; GM: Geometric mean; GSD: Geometric standard deviation; 95% CI: Confidence Interval, LB: Lower bound; UB : Upper bound; BDL: Below Detection Limit

Limit of detection (LODs): total arsenic (0.01 µg L^{-1}), As^{+5} (0.11 µg L^{-1}) and As^{+3} (0.37 µg L^{-1})
* Negative values due to very low log-transformed arsenic concentration, hence their GM and GSD could not be calculated.

Where negative values are given, it should be noted that they are in log and in actual represent low concentrations of arsenic.

Appendix 5.4. An overview of household and community level arsenic removal technologies (ARTs)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
Two Bucket Treatment Unit (2BTU)	Coagulation by addition of alum as a coagulant, potassium permanganate, added as an oxidizer, bind arsenic to the flocs, which are filtered out by sand layer at the bottom bucket.	1998	hh	60%	Bangladesh	Capital cost: USD 10 chemicals cost/year: USD15-20	.n.r	• 75% of the installed units removed arsenic to below 50 µg L^{-1}. • production from locally available material	issues in user's acceptability due to chemicals addition	Robinson (2000)
Three Kolshi Filter Unit (Adsorption and filtration)	Three traditional water filters or clay pitchers, stacked vertically in a frame. Top kolshi: contained a layer of iron filings and a layer of coarse sand, Middle kolshi: contains a layer of charcoal and a layer of fine sand, Bottom kolshi for the filtered water.	2000	hh	97%	Bangladesh	USD 40-50 capital cost	unit replacement after 3-5 years	• low cost and short term solution up for about 3-4 months • produced from locally available material.	• Solid lump formation after two weeks of usage and difficult to clean. • arsenic exceeds above 50 µg L^{-1}before 6 months	Munir et al. (2001); Centre for Affordable Water and Sanitation Technology (2009)
Rama Krishna Mission (RKM) Filter Unit (Coagulation and filtration)	Powdered Ferric Alum is used as coagulant in combination with bleaching powder solution as an oxidant. Tripura candle filter is used to filter Arsenic flocs.	1999	hh	Initially removes arsenic to below 0.05 mg L^{-1}	West Bengal	USD 40-58	n.r	easy to use and low cost	• poor arsenic removal due to issues with continuous supply of high-grade chemicals,	Robinson (2000)
Amal Domestic Water Purifier (Adsorption)	Composed of conventional two-chamber domestic candle filter body, with a layer of Aluminum oxide in the top chamber.	1998	hh	n.r	West Bengal	USD 40-58	Two years (claimed life of activated alumina)	adsorbing media can be regenerated by flushing with sodium hydroxide and acid.	• media saturation and clogging in less than 6 months	Robinson (2000)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
Kanchan Arsenic Filter (Adsorption)	Arsenic adsorbed on the rust of the iron nails. The rust and Arsenic flake off the nails, and are caught in the sand filter and retained	n.r	hh	85-95%	Bangladesh and India	USD12-40	More than 10 years	maintenance required at reduced flow rate	• Filter must be used almost every day to maintain the biological layer (maximum pause period is 48 hours). • Sand and iron nail selection and preparation are critical to ensure flow rate and treatment	Centre for Affordable Water and Sanitation Technology (2009)
Passive Sedimentation (Aeration)	Aeration of water for 12 hours and then leaving to settle for 12 hrs.	n.r	hh	30-50%	Bangladesh	USD 5	n.r	easy to use and short term household solution	long storage duration increases chances of faecal contamination	Centre for Affordable Water and Sanitation Technology (2009)
Tablet Reagents (Co-precipitation)	Handmade black coloured tablets made of ferric salt and activated charcoal	2000	hh	50%	Bangladesh	USD 2.00/year supply of tablets	n.r	higher arsenic removal efficiency of 95-100% in the lab with shelf life of 15 months	lower arsenic removal efficiency in the field	Das et al. (2000)
Sub-surface aerated water injection	Pumping the aerated water into the saturated zone of an aquifer, either through an abstraction point or an adjacent purpose-built well.	n.r	Comm	not efficient to remove arsenic below 10ug/L	Bangladesh	n.r	n.r	double-well designs have the advantage to use alternatively for arsenic removal	arsenic removal dependent on the groundwater properties such as; arsenic/iron ratio, effect of varying pH and	Matthews (2014) Van Halem et al. (2010)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
									interference by phosphorous.	
Alufloc	Household-level coagulant made of aluminium sulphite and ferric chloride	n.r	hh	98% with 100 µg/L	Bangladesh	USD 0.15 per bucket treated	n.r	effective in reducing arsenic content to safe levels	arsenic removal efficiency decreases with higher dissolved arsenic	Bedolla et al. (1999)
Stevens Institute technology (Coagulation, Sedimentation and Filtration)	Two buckets system: one for mixing the packet of iron coagulant and hypochlorite, the other one with sand bed to filter the flocs. Treated water is collected through a plastic pipe fitted with an outlet covered with a cloth filter to prevent sand	2001	hh	<50 ug/L	Bangladesh	n.r	n.r	enhanced coagulation and co-precipitation (ferrous sulphate) and less dependent on groundwater Iron	excessive bicarbonates may reduce the efficiency	Sutherland et al. (2001)
Safe water treatment unit (Coagulation and filtration)	300 litres upper reaction vessel filled with contaminated water and BAT solution, after 30 minutes of reaction time allowed to pass through sand filter to store into lower storage vessel	2004	Semi-comm	>95%	Pakistan	USD 400	4 years	• no longer contact time required • arsenic removal from 1000 µg L^{-1} to <10 µg L^{-1}	regular backwashing required	Kahlown et al. (2005)
Fill and draw treatment unit (Flocculation and filtration)	600 litres reaction vessel filled with water and the required quantity of oxidant and coagulant, stirred for 30 seconds and left overnight for sedimentation, filtered through sand bed and collected through vessel tap.	n.r	Semi-community type	n.r	installed in schools/colleges/communities in Bangladesh	USD 265/ unit	n.r	semi-community level option	longer contact time	Ahmed (2006)
Tube well-attached arsenic treatment unit (coagulation, sedimentation, and filtration)	Unit attached to hand pump-operated tube well, involved addition of sodium hypochlorite and alum in diluted form followed by	2000	community	90%	West Bengal, India	n.r	n.r	effective in removing 90% of the arsenic from tube well water	operation of the system depends on regular washing of the filter bed.	Ahmed and Rahman (2000)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
	mixing, flocculation, sedimentation, and up flow filtration in a compact unit									
Iron-arsenic treatment unit (precipitation and adsorption)	natural iron in water precipitated to remove arsenic by oxidizing As^{+3} to As^{+5} and finally by adsorption.	1998	both	50-80%	Bangladesh	n.r	n.r	reduction in arsenic from half to one-fifth of the original concentration.	community ownership created issues with regular washing of the filter bed	Ahmed (2006)
Combination of aeration, sedimentation & rapid sand filtration	medium-scale iron-arsenic removal plants	n.r	com m.	40-80% for arsenic level of 100 µg/L	Bangladesh	variable according to size	n.r	arsenic removal by co-precipitation and adsorption on natural iron flocs has good potential for arsenic content up to about 100 µg/L	higher water requirement for washing the filter beds	Ahmed (2006)
Arsenic removal by softening	Calcium carbonate formation by lime in water used to adsorb arsenic. arsenic removal through sorption of arsenic onto magnesium hydroxide solids that form during softening.	n.r	both	40-70%	Multiple regions	n.r	n.r	efficient to treat water with high hardness, especially at pH >10.5.	large lime doses (800–1,200 mg L^{-1}) result in large volume of sludge. pH adjustment of treated water required, relatively low removal efficiencies	McNeill and Edwards (1997)
Activated alumina filters (BUET activated alumina, Alcan enhanced activated alumina and Apyron Arsenic treatment units)	Adsorption of arsenic on active surface of the media	n.r	hh to sem-com munit y level	moderate efficiency	Bangladesh and India	n. r	6 months	• no chemicals required • highly selective towards As^{+5} • effective with water with high total dissolved solids (TDS)	• with exhaustive sorptive sites media cannot remove arsenic • interference by iron and phosphate	Ahmed (2006)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
									• 5–10% of the alumina is lost during removal process and the capacity of the regenerated medium is reduced by 30–40%. • replacement of activated alumina after 3–4 regenerations.	
Activated aluminium hydroxide hydrogel	Hydrogel produced from hydrated aluminum sulfate, powdered calcium hypochlorite, ammonium hydroxide and demineralized water.	1994	n.r	>90%	Tucuman province (Argentina)	n.r	n.r	arsenic reduction (40–800 µg L^{-1}) to below 10 µg L^{-1}	not found	Litter et al. (2012)
Granular iron oxide (Bayoxide®, GFO)	contains less than 70% of Fe_2O_3	1999	com m	95%	Multiple regions	n.r	n.r	viable product with arsenic removal efficiency	interferences of other ions during arsenic adsorption	Dennis (2016)
Granulated ferric hydroxide e.g. granular ferric hydroxide GFH® or (AdsorpAs®)	Arsenic removal by activated alumina controlled by the pH and arsenic level of water, Arsenic removal is optimum in the narrow pH range from 5.5 to 6.0 when the surface is positively charged.	n.r	both	>90%	India and Bangladesh	USD 4,300 for community	>3,600 litres of arsenic free water per day for 100 families	• highly effective adsorbent for As^{+5} and As^{+3} • adsorption capacity of 45 g/kg for arsenic on a dry weight basis	• requires aeration for oxidation of water and pre-filtration for removal of iron flocs before filtration	Pal (2001) Matthews (2014)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
									through active media • regeneration of saturated alumina results in high-arsenic-contaminated caustic waste water.	
Electro-Chemical Arsenic Remediation (ECAR) (electro-coagulation)	Uses a small electrical charge through an iron electrode to produce ferric hydroxides, oxy-hydroxides, and oxides, a form of rust. The rust reacts with the arsenic in the water to be filtered or allowed to settle out of the water.	n.r	both	>90%.	Argentina Bangladesh	n.r	n.r	• does not require continuous chemical supplies • electrode cleaning by reverse current once a day.	electricity dependent option	Matthews (2014)
The Shapla Arsenic Filter (Adsorption)	Iron-coated brick chips manufactured by treating brick chips with ferrous sulphate solution used as adsorption media	n.r	hh	80-90%	Bangladesh.	capital cost: USD10 media replacement cost/year: USD10-15	media lifespan of 3-6 months)	used filter media is non-toxic and can be disposed of safely	n.r	Centre for Affordable Water and Sanitation Technology (2009)
READ-F Arsenic filter (Ion-exchange resins)	the READ-F is ethylene-vinyl alcohol co-polymer-borne hydrous cerium oxide (an adsorbent)	n.r	hh	>95%	Bangladesh and Japan	USD 50-70	3 years	• effective adsorption of As^{+5} and As^{+3} • regeneration by adding sodium hydroxide and then Sodium hypochloride and finally washing with water	pre-treatment of iron by sand filtration to avoid clogging of the resin bed.	Matthews (2014)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
SORAS (solar oxidation and removal of Arsenic)	Based on principle of SODIS but lemon juice is added and kept under sunlight as a source of UV to cause oxidation of As^{+3} to As^{+5}. The As(V)/$Fe(OH)^3$ co-precipitate and settles at bottom.	n.r	hh	75- 90%	South East Asia, Latin America	minimal	na	reactive oxidants are produced photo chemically with sunlight	low scalability	Centre for Affordable Water and Sanitation Technology (2009)
SAFI filter (adsorption & filtration)	Removes arsenic by filtration and adsorption through porous material of filter.	n.r	both	>73%	Bangladesh	46 USD	n.r	user friendly and readily available	reduced flow rate of water with the passage of time	Rahman et al. (2005)
Memstill® technology	combines multistage flash and multi-effect distillation modes into one membrane module	2007	hh	n.r	Bangladesh and India	n.r	n.r	• arsenic free water at cost lower than for reverse osmosis (RO) and distillation • Small scale applications using solar heat	improper cleaning of membrane may results in expiry of membrane	Feenstra et al. (2007)
Cerium oxide	CeO_2 nanoparticles firmly fixed on the walls of silica monoliths(SCO) and demonstrated a superior dynamic arsenic removal performance	2012	both	87%	Multiple regions	n.r	n.r	SCO composite easily desorped/regenerated for re-use	n.r	Toshio Shimoto (2007)
Magnetic micro-sorbents	the high saturation magnetization of Fe_3O_4@TiO_2nanoparticles (45.56 emu/g) facilitates their separation from aqueous solutions by use of a moderate magnetic field and cause Arsenic adsorption	2003	both	n.r	n.r	n.r	n.r	faster adsorption of As^{+3}	tremendous application in water industry and no drawbacks found in literature	Lan (2015)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
Nano-particulate ZVI(NZVI) (Adsorption)	rapid removal of As^{+3} and As^{+5} from subsurface environment	2005	both	99.9%	Multiple regions	n.r	variable	formation of arsenic neutral after reaction of As^{+5} and As^{+3} on the nano-particle surface.	efficiency decreases by increasing pH and arsenic concentration in solution	Ramos et al. (2009)
Ion exchange media	Resin made of cross-linked polymer skeleton having attached the charged functional groups through covalent bonding. Following pre-oxidation of As^{+3} to As^{+5} is removed is removed using the ion exchange process.	n.r	both	>90%	Multiple regions	USD 2,000.	variable	• effective technology even at higher flow rates of tube well water. • As^{+5} removal is relatively independent of pH and influent concentration.	• excess oxidant may damage the resin and thus needs to be removed. • Interference by competing anions to affect run length. • clogging by suspended solids and precipitated iron	Clifford (1999)
Nano-filtration	Separation of ionic species by nano-filtration membrane is dependent on membrane charge and pore size	n.r	both	95% of As^{+5} and >75% of As^{+3}	Multiple regions	variable	n.r	high pressure, high pH and low temperature favor more efficient arsenic removal.	• fouling or scaling of membrane by iron or manganese • backwashing cannot recover membrane fouling	Sato et al. (2002)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
									• As^{+3} cannot be removed	
Reverse Osmosis (RO)	high-pressure membranes of RO (75–250 PSI or higher) causes reversal of natural osmotic flow resulting in rejection of polyvalent ions including arsenic oxy-anions	n.r	both hh and com m	40-99%	Argentina, e.g. in the provinces of Santa Fe, Córdoba and La Pampa	variable with size	n.r	• simple operation and maintenance (O&M) as no chemical addition • periodic membrane cleaning required • effective for community and household application • effective for treating water with high total dissolved solids (TDS) water	• water recovery rates of only 10–20% • higher electric power consumption • higher capital and operating costs • higher risk of membrane fouling • suitable for lower levels of arsenic • disposal of arsenic containing rejected brine water/sludge is a concern • poor removal of As^{+3} as oxidation to As^{+5} is difficult and may cause membrane damage • pre-treatment required	Clifford (1999); Litter et al. (2010); Robert (2002)
Capacitive Deionization (CI)	unit consists of low-cost filter of coal electrodes causes deionization by flow	n.r	both	98.51%	Mexico	n.r	n.r	• system cleaning with smaller amount of chemical reagents	suitable for water with total dissolved solids	Litter et al. (2010) Garrido et al. (2008)

ART name	Process/Removal mechanism	Year	Type	Removal efficiency	Region of Application	Cost (unit & yearly operation and maintenance (O&M)	Claimed life	Advantages	Drawbacks	Reference
	through a capacitor with electrostatic load							• removal of As^{+5} and As^{+3} • rejection of 3-4% of treated water • lower operation and maintenance (O&M) cost	(TDS) <3000 mg L^{-1}	
Electrodialysis	Electrodialysis is a membrane process, during which ions are transported through semi permeable membrane, under the influence of an electric potential	n.r	com m	80%	Multiple regions	n.r	n.r	• equally effective like RO in treating high total dissolved solids (TDS) water • reduced scaling	• very high costs • pre-treatment required	Litter et al. (2010)
In-situ remediation: Permeable Reactive Barriers(PRB)	Appropriate reactive material based on based on sorption, precipitation, chemical reaction and/or biogenic reactions, is able to induce physicochemical and/or biological processes to remediate groundwater contamination	1999	com m	n.r	Multiple regions	n.r	n.r	• significant cost benefits • low operational costs • low-cost local materials can be used	• efficiency affected by microbiologic al and geochemical processes • corrosion of materials. • diminished permeability by precipitation of sulfides, oxides, hydroxides and carbonates.	Litter et al. (2010)

hh: household, comm: community, USD: US Dollar, n.r: not reported

Appendices-Chapter 8

Appendix 8.1 Record of Skin Lesions

Part-E: General Health Observations Form

Date		Village	
House-ID		Person ID	
Body weight		Occupation	
Smoking status	□ Yes □ No		

Observations

Observations	Answers	Detail
Has a doctor ever told you that you have the following disease? □ Cerebovascular disease (Stroke) □ Parkinson's disease / Dementia □ Heart diseases □ Hypertension □ Chronic bronchitis / Pulmonary emphysema □ Asthma □ Pneumonia (type: _____) □ Pulmonary tuberculosis (TB) □ Intestinal ulcer □ Diabetes mellitus □ Arthritis □ Osteoporosis □ Mental disorder(s) (type: _____) □ Cancer (type: _____) □ Infertility □ Miscarriages □ Other disease (specify :_____)	□ No □ Yes, I found out within last one year □ Yes, I have it for years □ Do not know □ Refused	
Observations	**Answers**	**Detail**
Presence/Absence Pigmentation changes, skin lesions and hard patches on the palms and soles of the feet (hyperkeratosis) ***(Initial screening)***	□ Yes □ No	
Hyperpigmentation: Raindrop-like spots Diffused dark brown spots or darkening of the skin on the limbs or chest, back, and abdomen. ***(Final Screening by Physician)***	□ Yes □ No	
Keratosis: Thickening of the skin of the palms of hands or the soles of feet, or small flanges (0.4 to 1 cm in diameter) mall corn-like elevations on palms and soles. ***(Final Screening by Physician)***	□ Yes □ No	
Persistent vomiting, abdominal pain and	□ Yes □ No	

diarrhea		
Bladder cancer	□ Yes □ No	
Lungs cancer	□ Yes □ No	
Any other cancer type	□ Yes □ No	
diabetes	□ Yes □ No	
cardiovascular disease	□ Yes □ No	
hypertension	□ Yes □ No	
Other_____	□ Yes □ No	
Signature (non-physician health workers)		
Signature (Physician)		

Biomarker Samples

Hair	□ Yes	□ No	□ Refused
Nail	□ Yes	□ No	□ Refused
Urine	□ Yes	□ No	□ Refused
Samples Collected by (team member)		Verified by (team member)	

Appendix 8.2: Summary of quality control data of six analytical batches of urine samples

(a): QC results of MDLs, MRLs, DUP and MS

Parameter	Method Detection Limits (MDLs)		Method Reporting Limits (MRLs)		Duplicate (DUP)				Matrix Spike (MS)			
	mean	SD	mean	SD	% RPD	SD	% Rec.	SD	% RPD	SD	%Rec.	SD
Total As	0.13	0.22	0.27	0.49	4.2	3.2	93.0	11.3	1.40	1.42	99.16	20.69
AsIII	0.12	0.05	1.01	0.01	7.4	7.7	97.7	12.1	2.80	2.08	96.48	19.66
AsV	0.32	0.43	2.51	3.67	6.6	9.9	98.0	10.0	0.25	0.21	104.71	16.94
MMA	0.20	0.11	1.18	0.01	4.5	4.7	102.3	12.9	1.87	1.21	-	-
DMA	0.13	0.04	1.05	0.01	4.7	5.7	104.0	14.1	0.20	0.00	99.84	20.79
AsB	0.14	0.05	1.04	0.02	7.1	7.8	−	−	−	−	102.48	14.08

(b): QC results of MSD, SRM and BS

Parameter	Matrix Spike Duplicate (MSD)				Standard Reference Material (SRM) NIST 1640a		Laboratory Fortified Blank (BS)	
	%RPD	SD	% Rec	SD	% Rec.	SD	% Rec.	SD
Total As	2.18	1.52	99.16	20.39	95.17	6.71	−	−
AsIII	1.43	1.12	96.60	20.00	−	−	100.33	7.81
AsV	1.96	1.60	124.80	229.40	−	−	100.00	10.41
MMA	0.70	0.47	-	-	−	−	83.50	0.71
DMA	1.22	0.97	99.50	21.00	−	−	102.17	8.86
AsB	11.00	7.07	102.75	13.62	−	−	92.50	15.07

SD: Standard deviation; % Rec: Expected percent recovery: 75-125% , % RPD: Expected relative percent difference: <25%
Minimum limits of detection; tAs (0.01 µg L^{-1}), AsV (0.10 µg L^{-1}), AsIII (0.10 µg L^{-1}), MMA (0.12 µg L^{-1}), DMA (0.10 µg L^{-1}), AsB (0.10 µg L^{-1})

Appendices-Chapter 9

Appendix 9.1: A summary of average daily dose (ADD) and life time average daily dose (LADD) of arsenic and its species

Arsenic analyte	ADD (μg kg^{-1} day^{-1}) (mean ± SD) (mean ± SD)				LADD (μg kg^{-1} day^{-1}) (mean ± SD) (mean ± SD)			
	Water	Rice	Wheat	Total	Water	Rice	Wheat	Total
As(III)	0.274±0.682	0.134±0.075	0.518±0.313	0.926±0.770	0.101±0.293	0.050±0.044	0.192±0.177	0.342±0.385
As(V)	13.177±29.689	0.050±0.016	0.430±0.167	13.656±29.698	4.847±13.030	0.019±0.013	0.159±0.119	5.024±13.058
iAs	13.45±30.37	0.184±0.082	0.947±0.463	14.582±29.650	4.948±13.32	0.069±0.054	0.351±0.289	5.367±13.087
DMA	0.018±0.006	0.028±0.014	0.004±0.001	0.051±0.018	0.007±0.005	0.011±0.009	0.002±0.001	0.019±0.014
MMA	0.013±0.004	0.001±0.000	0.004±0.001	0.018±0.005	0.005±0.003	0.000±0.000	0.002±0.001	0.007±0.005

Appendix 9.2: Lifetime (cumulative) species specific cancer and non-cancer risk from water and staple food

Arsenic analyte	Non-cancer (mean±SD)				Cancer risk (mean±SD)			
	Water	Rice	Wheat	Combined	Water	Rice	Wheat	Combined
As(III)	61.971±139.047	6.920±15.108	3.623±62.602	192.514±157.977	0.00923±0.02303	0.00400±0.00329	0.01518±0.01316	0.02841±0.02908
As(V)	51.508±102.734	0.180±0.079	1.566±0.725	53.254±102.906	0.00936±0.02045	0.00003±0.00002	0.00028±0.00017	0.00968±0.02049
DMA	0.001±0.001	0.002±0.001	0.0002±0.0001	0.003±0.002	1.2E-07±7.7E-08	1.9E-07±1.3E-07	2.7E-08±1.6E-08	3.5E-07±2.1E-07

Appendix 9.3: Lifetime (cumulative) non-cancer risk due to iAs intake from water and staple food *at 95% CI* posed to male and female population of the study villages

Population	Statistics	Chak-46	Chak-48	Chak-49	Basti Balochan	Badarpur	Kotla Arab
Female	Mean	41.66	268.21	225.29	30.01	487.46	37.59
	LB	24.41	18.06	64.61	18.77	369.56	22.28
	UB	58.91	518.36	385.97	41.24	605.36	52.90
Male	Mean	53.35	115.30	204.74	79.57	366.33	50.91
	LB	40.49	60.47	117.69	47.49	258.82	27.32
	UB	66.21	170.13	291.79	111.64	473.84	74.49
All participants	Mean	49.12	129.46	209.44	45.77	417.72	42.88
	LB	38.80	74.23	133.43	31.47	336.67	29.74
	UB	59.43	184.68	285.44	60.08	498.77	56.01

Cumulative non-cancer skin lesions risk initiating at the current age of participant up to 67 years of total life

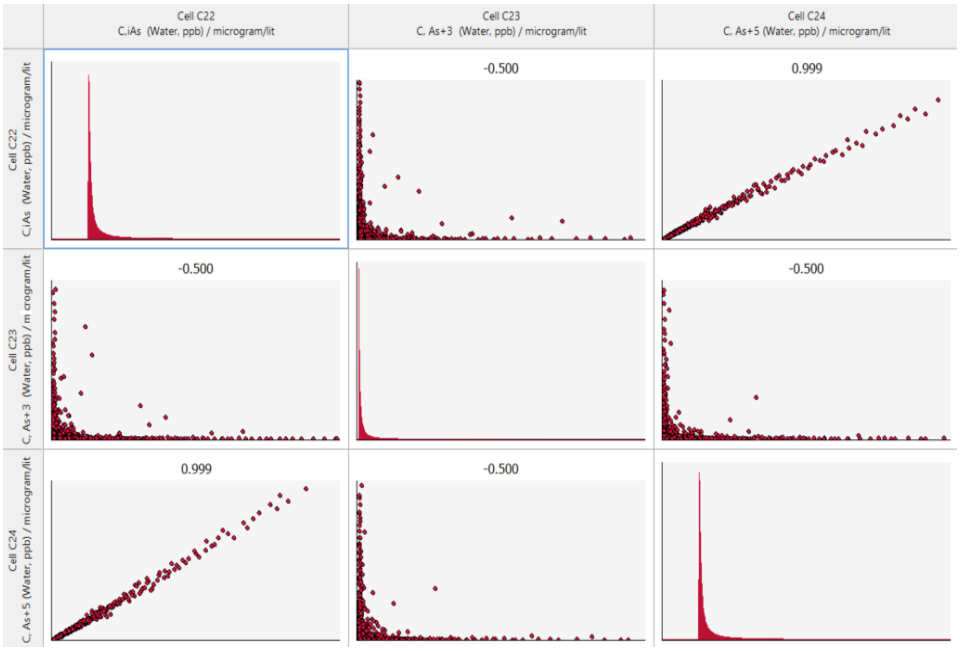
Appendix 9.4: Table- RS-3: Lifetime (cumulative) cancer risk due to iAs intake from water and staple food *at* 95% CI posed to male and female population of the study villages

Population		Chak-46	Chak-48	Chak-49	Basti Balochan	Badarpur	Kotla Arab
Female	Mean	0.008357	0.118234	0.040060	0.005055	0.079348	0.006616
	LB	0.005074	0.087702	0.007341	0.002882	0.038391	0.003915
	UB	0.011641	0.324170	0.072780	0.007229	0.120305	0.009316
Male	Mean	0.010591	0.022727	0.030152	0.012284	0.085334	0.008177
	LB	0.007801	0.010139	0.010526	0.006392	0.049354	0.004379
	UB	0.013381	0.035316	0.049779	0.018175	0.121314	0.011974
All participants	Mean	0.009782	0.031571	0.032417	0.007355	0.082795	0.007235
	LB	0.007641	0.009613	0.015612	0.004801	0.056160	0.005026
	UB	0.011923	0.053529	0.049222	0.009909	0.109429	0.009445

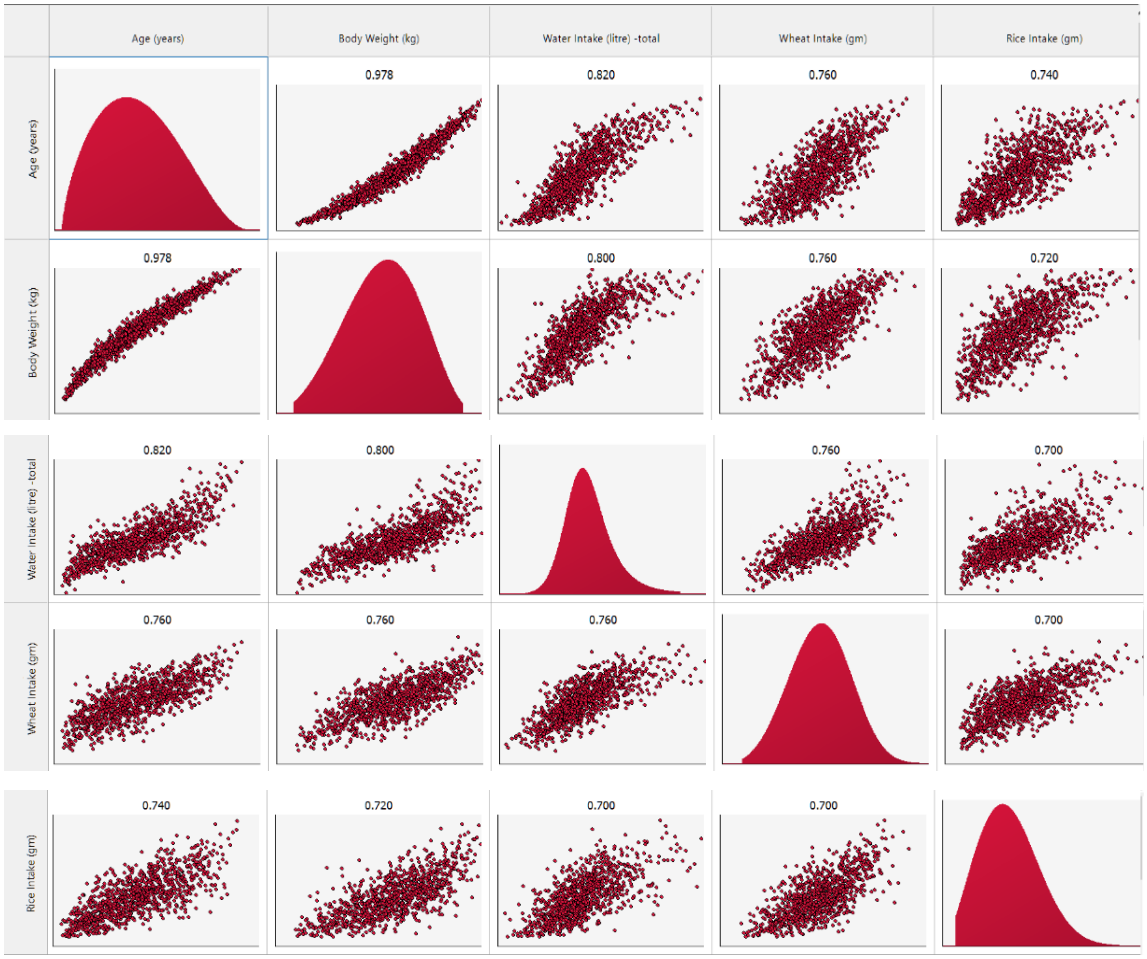
Cumulative skin cancer risk initiating at the current age of participant up to 67 years of total life

Appendix 9.5: Lifetime excess lung and bladder cancer risk estimates (per 10,000 populations) posed to male and female population of the study area

Cancer type	Statistics	Chak-46	Chak-48	Chak-49	Basti Balochan	Badarpur	Kotla Arab
Bladder (95% CI)							
Female	Mean	17	65	17	6	431	4
	LB	14	37	15	5	288	3
	UB	19	92	19	6	574	6
Male	Mean	13	52	13	4	345	3
	LB	11	30	12	4	231	2
	UB	15	74	15	5	460	5
	Total	30	117	30	10	777	8
Lung (95% CI)							
Female	Mean	2	8	2	1	52	1
	LB	2	5	2	1	35	0
	UB	2	11	2	1	70	1
Male	Mean	1	5	1	0	33	0
	LB	1	3	1	0	22	0
	UB	1	7	1	0	43	0
	Total	3	13	3	1	85	1
	Overall	33	129	34	11	862	9



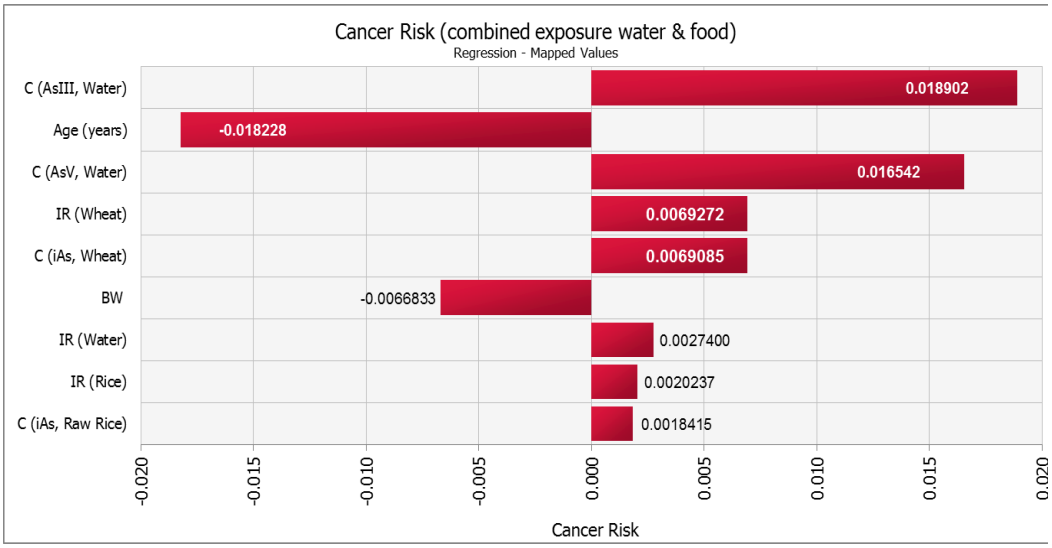
Appendix 9.6: Copula-1 (Elliptical t-type) output for modelling dependence of As species in water (iAs, AsIII and AsV)



Appendix 9.7: Copula-2 (Elliptical t-type) output for modelling dependence of age, body weight, daily intake of water, wheat and raw rice

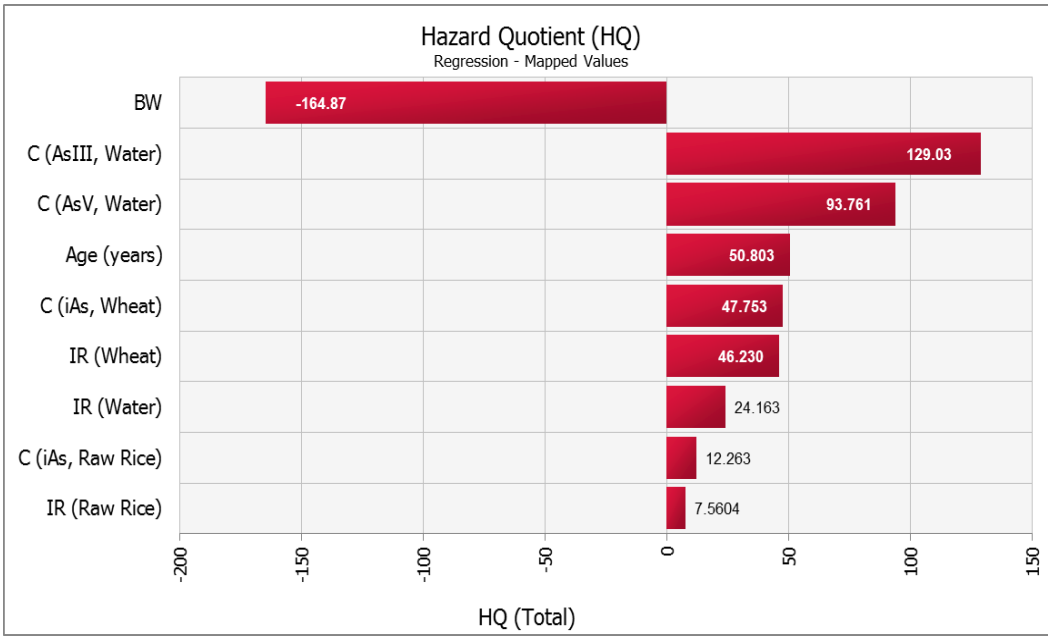
Appendix 9.8. Sensitivity analysis of Probabilistic risk estimates

Sensitivity analysis for skin related probabilistic health risk due to iAs, AsIII, AsV and DMA based on mapped values presented as regression coefficient (Appendices 9.9 and 9.10). For every 1SD increase in AsIII and AsV concentrations in water (SD 9.74 µg L^{-1} and 428.59 µg L^{-1}) and wheat intake rate (SD 196 g day^{-1}), the cumulative cancer risk increased by 1.9%, 1.7% and 0.7% respectively (Appendix 9.9). For every 1SD increase in age (SD 15.6 years), the total cumulative cancer risk of iAs decreased by 1.82%.



Appendix 9.9: Sensitivity Analysis for Simulated Cumulative Cancer Risk - Tornado Plot

Mapped values presented as regression coefficient (Appendix 9.10) showed that AsIII and AsV concentration in water have significant positive correlation with HQ and for every 1SD increase in AsIII and AsV concentrations, the HQ increased as 129.03 and 93.761 respectively. With 1SD increase in body weight (19.13 kg), a decrease of 164.87 in HQ of iAs was observed. This implies that for adults with increasing body weight HQ decreased, whilst increased for children. Since age was directly correlated with BW, thus both were expected to behave in similar pattern.

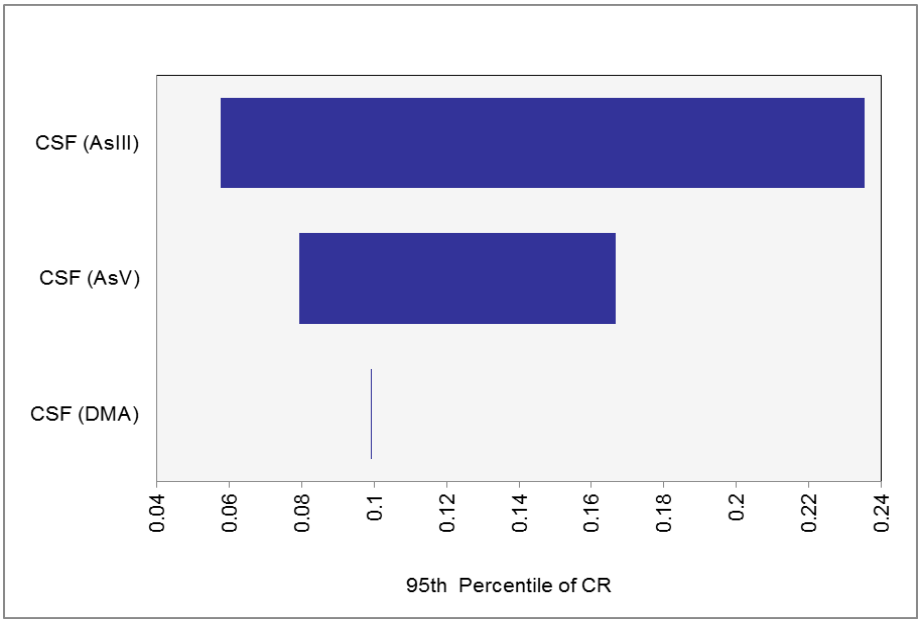


Appendix 9.10: Sensitivity Analysis for Simulated Non-Cancer Risk (HQ) - Tornado Plot

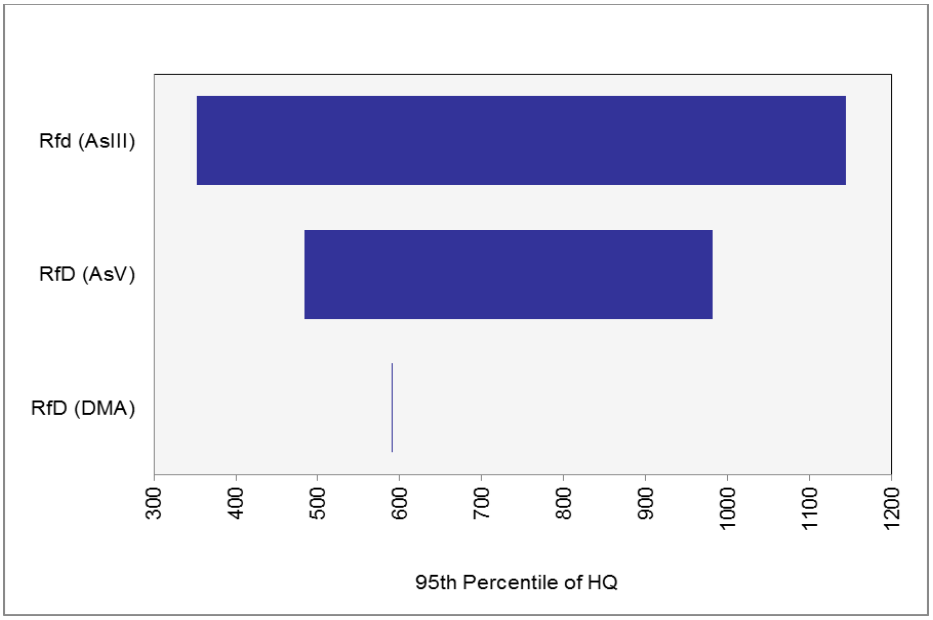
Appendix 9.11. Uncertainty Analysis for Cancer and Non-cancer risk

The uncertainty analysis was carried out using 'Advanced Sensitivity Analysis' of @Risk using an uncertainty factor of 3 to iAs RfD (United States Environmental Protection Agency, 2011) and 100 for DMA (Agency for Toxic Substances and Disease Registry, 2007) in 7 incremental steps each consisting of 5000 iterations to have total of 35000 iteration for each uncertainty factor. These uncertainty factors were applied separately on base values of both RfDs and CSFs to assess the uniform distributions (minimum-maximum) for the iAs and AsV (RfD: 0.0001-0.0009, CSF: 0.5-4.5), AsIII (RfD: 0.0000017-0.000015, CSF: 25-225) and DMA (RfD: 0.0002-2, CSF: 0.00015-1.5).

The 95th percentiles simulated cumulative cancer risk (Appendix 9.12) with min-max values for uncertainty bounds for CSF of AsIII, AsV and DMA were 0.1000 (0.0574-0.2355), 0.1004 (0.0792-0.16667), 0.0990 (0.0990-0.0990) respectively. This shows a variation from baseline CSF in cancer risk as 0.1781 (-43 to 136%), 0.0876 (-21 to 66%), 0.0 (0% from baseline CSF) for AsIII, AsV and DMA respectively.



Appendix 9.12: Sensitivity analysis for species specific simulated cancer risk using uncertainty bounds of CSF



Appendix 9.13: Sensitivity analysis for species specific simulated non-cancer risk using uncertainty bounds of RfD

The 95th percentiles simulated HQ with min-max values for uncertainty bounds (Appendix 9.13) for RfD of AsIII, AsV and DMA were 581.75 (351.97–1144.24), 583.70 (483.38–981.58), 590.346 (590.375–590.104) respectively. This shows a variation from baseline RfD in non-cancer risk as 792.27 (-39 to 97%), 498.20 (-17 to 68%), 0.271(-0.04 to 0.005%) for AsIII, AsV and DMA respectively.

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